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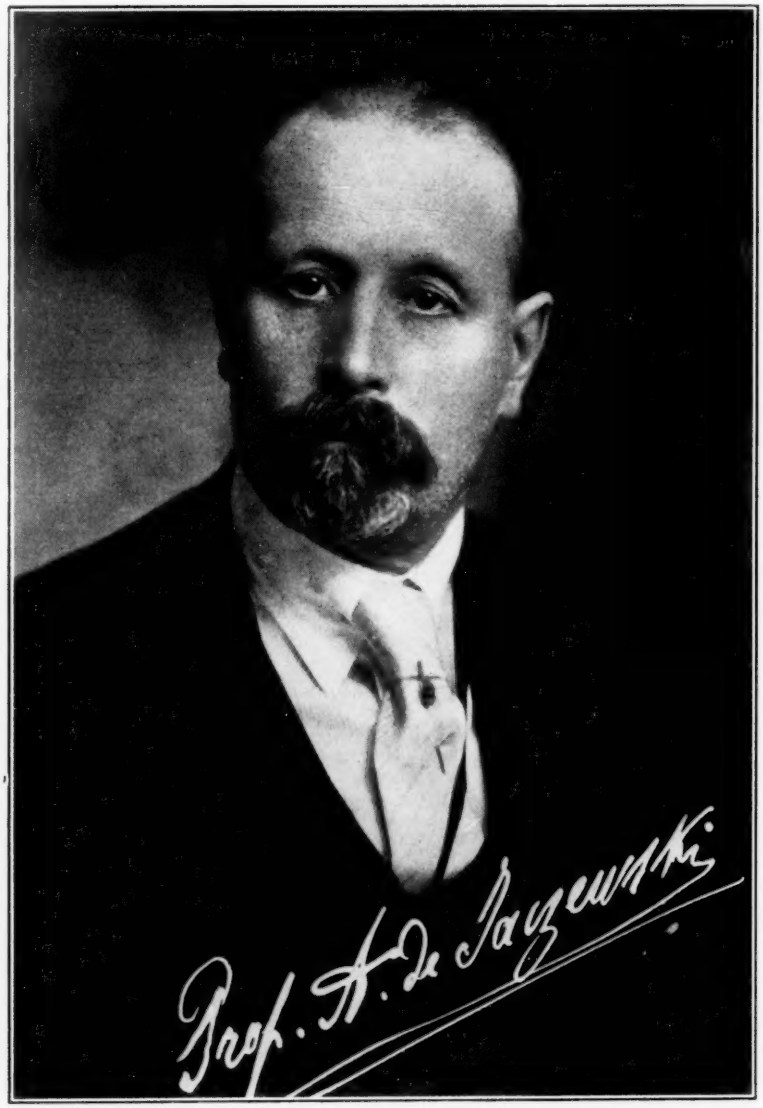

ARTHUR JACZEWSKI

UNIV. OF

PHYTOPATHOLOGY

VOLUME 23 NUMBER 2

FEBRUARY, 1933

ARTHUR JACZEWSKI

1863–1932

L. R. JONES[1]

Professor Arthur Jaczewski, eminent Russian mycologist and plant pathologist, completed his labors and was laid to rest February 12, 1932. For over 40 years Jaczewski had been a tireless contributor to Eurasian mycology and for 3 decades the recognized leader and teacher in Russian phytopathology. His earlier efforts culminated in the reconstruction period following the World War. He then reorganized and was the director of the laboratory for mycological and phytopathological researches in Leningrad, which later became one of the present group of national institutes, "Institut Mycologique et Phytopathologique de Jaczewski." This now continues as the central institute in this field for all U. S. S. R. Following his death his institutional coworkers prepared a memorial statement the substance of which has been incorporated in the present article.

Arthur Jaczewski was born November 3, 1863, at the family country seat,[2] "Rylkowa," in the former Russian province, Smolensk, westward of Moscow. Much of his youth was there passed with his parents in quiet rural surroundings. There he enjoyed the natural incidents of such country life, happy walks in field or forest, along with riding and hunting. Thus he

[1] The nominal writer of this sketch wishes to acknowledge the cooperation of several of Professor Jaczewski's American friends. C. L. Shear helped especially concerning Jaczewski's mycological work. Aid also has come from H. L. Bolley, H. S. Fawcett, and J. G. Dickson, who, in 1903, 1923, and 1930, respectively, like the writer, in 1930, had the privilege of visiting the Russian laboratories or experimental fields. Michael Shapovalov, also, furnished certain helpful data on Russian conditions and scientific literature. We are, however, above all, indebted to his widow, Madame Catherine Jaczewski, who with their one son, Peter, survives him. To them is owing a translation of the memorial prepared by the coworkers of the Jaczewski Institute. The son, Peter, received his later technical training in this Institute, of which he continues a staff member. He was closely associated with his father's later researches and writings.

[2] As visited by Bolley in 1903, this was still a great princely estate of the old Russian type, for the family of Arthur de Jaczewski, as the name was then written, were of the old Russian nobility. But his scientific training had strengthened his natural democratic character. He already dreamed of a future republic. He was contributing as he might in personal relations to the kindly uplift of the peasantry of the family estate and through his scientific efforts to the general betterment of rural conditions especially by the applications of modern methods of agricultural science.

early learned to love and study nature in all her forms, especially flowers and trees. The foundations were securely laid there for his later life work. As a boy he began systematic study of the native plants and started an herbarium. Quite as influential, perhaps, in the later trends was the familiarity then acquired with the crop plants of the great fields, the orchards, and gardens of Rylkowa. As was common in such early Russian families, French was the familiar home language. It was the more natural, therefore, that he spent much time in early life in France and Switzerland. Here as a youth he made long Alpine excursions, observing the plants closely. These prepared the way for his later intensive botanizing with Swiss mycologists. His interest thus became so keen, as Madame Jaczewski tells us, that "in the year 1892 he founded at the Rochers de Nage, above Montreux, the Jardin Alpin Rambertia."

His advanced studies were at the University of Berne, Switzerland. The epochal work of Woronin had naturally directed the attention of all Russian scientists to the fascinating and economically important problems of mycology. It was, however, the inspiring leadership of his Berne professor, Eduard Fischer, that contributed to young Jaczewski's early scientific advancement in special studies upon the fungi. His esteem for his early master continued to influence all his later work. In this association he began his mycological publications in Switzerland in 1889, and in 1890 his "Erysiphaceae de la Suisse" appeared. Although specializing for some years in his researches upon the mycological flora of Switzerland, he also attended special lectures at the Sorbonne and the Universities of Lausanne and Montpellier. He soon visited Italy and made an excursion into Algeria upon the mycology of which he published in 1893. In this year, also, appeared the first of his long series of papers upon Russian mycology, naturally beginning with his home province, in a catalogue of fungi of the "Gouvernement de Smolensk." In his early studies he became especially interested in the Pyrenomycetes and made a thorough study of the species occurring in Switzerland. He published in 1894–95 upon a number of different families of this group, including the Xylariaceae, Capnodiaceae, and Massariaceae, along with a more general essay on the natural classification of the Pyrenomycetes.

In 1895, in association with Professor Tranzschel, he began the distribution of "Fungi Rossiae Exsiccati." Seven fascicles of this, 350 numbers, were issued. In 1896 Jaczewski transferred his more intensive work to the Botanical Garden of St. Petersburg, there initiating a laboratory of mycology and plant pathology. With him he brought an excellent personal library and herbarium. His permanent return to Russia at the end of the last century marks the natural broadening of Jaczewski's interests concerning the bearings of mycological science upon Russian agriculture.

He had, during the summers on the Smolensk family estate, continued in intimate personal contact with rural life and agricultural problems. He held liberal political views and was of an eager progressive temperament socially as well as scientifically. It was, therefore, the natural thing that, about 1900, he recognized the great opportunity and need for organized development of plant pathology in Russia. He found the National Ministry of Agriculture sympathetic, and, in 1901, there was officially founded under this Ministry "The Central Laboratory of Plant Pathology."[3] Of this, Jaczewski was the scientific organizer and official chief. It was located in the Jardin Botanique, which, at the outset, afforded helpful library and laboratory space and other facilities.

From the first he was interested in three types of service in which this laboratory might meet the needs of all Russia. His first purpose was the continuation of his work upon the mycological survey of Asiatic, as well as European, Russia. Reference has already been made to the nature and

[3] For the details of these early developments of the "Central Laboratory" we are indebted chiefly to Jaczewski's own account: La laboratoire central de pathologie végétale du Ministère de l'Agriculture à St. Petersbourg. Bul. Soc. Mycol. France 19: 324–329. 1903.

For a more comprehensive review of the early developments in this field, the writer has relied upon the cooperation of Michael Shapovalov, of the Rubidoux Laboratory, California. Shapovalov bases the following summary upon an 18-page pamphlet (in Russian) by Jaczewski: St. Petersburg, 1911, entitled (transl.) "A sketch of the status and development of phytopathology in Russia."

This cites the few isolated Russian papers that antedated the organized developments. These included those of Woronin (1868 and later), S. M. Rozanov (1871), and the Phylloxera control committees (1880). In 1894 under the Ministry of Agriculture was organized a Bureau of Applied Botany. Under this, among other activities, was included some regional mycological surveys by Sorokin, Varlich, Dekkenbach, and Jaczewski. This decade saw notable increase in Russian publications relating to mycology and phytopathology. He cites these as follows: N. Pal'chevski (1891), Sorokin (1892), I. P. Borodin (1897), G. Varlich (1897–8), S. Rostovsev (1899), Dekkenbach (1899–1900), N. Speshnev (1900), Jaczewski (1897–99). The founding of the "Central Laboratory" came in 1901, with increased and varied activities as noted above. Jaczewski was at first director but resigned in 1905, being succeeded by A. A. Elenkin. In 1907 a new "Bureau of Mycology and Phytopathology" was formed under the Ministry of Agriculture of which Jaczewski was the director, with G. N. Dorogin and E. A. D'iakonova as associates. Especial attention was given to building up the library and herbarium that by 1911 was stated to rank with the best in Europe.

Shapovalov adds that this Bureau continued until the Revolution. Then, in 1917, it became the Division of Mycology and Plant Pathology.

Madame Jaczewski advises in correspondence that, in 1909, Professor Jaczewski made a gift of his personal library and herbarium to the bureau of which he was director. However, during the vicissitudes of the succeeding 2 decades of his life he continued to care for and enrich these from his private funds as supplementing public support, and these now constitute the basis of the valuable collections of the Jaczewski Institute, Leningrad.

importance of his continued contributions to this. The second aimed to combine especial attention to the parasitic fungi of the Russian economic plants and the related phytopathological problems, including control measures. His third concern was for educational work with the agricultural public in general, but, especially in later years, the encouragement and training of the younger Russian phytopathologists. To these purposes he faithfully devoted his time and means for the remaining 30 years of his life. His fidelity to his scientific ideals, regardless of the endless distractions and terrific strains of the wars and revolutions, is an example not to be forgotten. The size of the tasks he outlined is suggested when one recalls that, in area, the Russian territory includes 1/6 of the earth's surface and in diversity her efforts at plant culture extend from the Arctic Circle to the Caucasus and from the shores of the Baltic and Black Seas across Siberia to those of the Pacific. Yet, with tireless energy and firm grasp of detail he grappled with these duties. In 1903 Professor Bolley found him directing plant-pathological work in various regional experiment stations, and this was continuing with an able staff of associates at the time of the writer's visit in 1930. Rarely has like need found fitter scientific leadership in public service. In 1907 the earlier movement culminated, again under his direction, in the organization of the Bureau of Mycology and Phytopathology. The detailed changes in organization and personnel are shown in the preceding footnote. In this bureau were combined both research and advisory services manned by an adequate staff. Lectures in phytopathology, also, were given by Professor Jaczewski and his associates in the Institutes of Forestry and of Agronomy. These developments necessarily took much of his time and energy. Nevertheless, he continued with dauntless zeal his earlier lines of personal mycological work. While maintaining and enlarging the St. Petersburg herbarium and library, he also continued to work much during the summer at Rylkowa. Here were his simpler private laboratory and collections. In some respects his official relations enabled him, indeed, to enlarge and broaden, as well as intensify, his mycological undertakings. Immediately upon the establishment of the "Central Laboratory" in 1901 he began the preparation of a monographic treatment, 2 volumes of which were published, upon the mycological flora of European and Asiatic Russia. In 1913–17 he prepared and published in Russian 2 volumes on the determination of the fungi, containing keys and illustrations of genera and descriptions of the species. To the revision and enlargement of this he devoted time throughout the remaining years of his life. Two months before his death appeared the first part of the third edition of this work, covering the Phycomycetes. Besides such more inclusive works, he published many special articles on fungi and was very deeply interested in the problems of phylogeny. His latest paper on this

subject was published in Phytopathologische Zeitschrift in 1929. Professor Jaczewski was a man of unusual powers of memory and had a wide and thorough acquaintance with nearly all groups of the fungi. He held conservative ideas concerning genera and species and his leadership has laid sound foundations for future developments of mycology in Russia.

Meanwhile numerous publications, including manuals, appeared treating directly of phytopathology especially as applied to the special crops and conditions in Russia. From the outset of his personal leadership in Russian plant pathology to his very last days he strove ceaselessly to serve his country through science and to inspire his numerous pupils to do likewise.[4] It is unnecessary to dwell upon the hardships that he, like many of the Russian contemporaries, experienced during and after the Revolution. Perhaps the solace of determined work helped to sustain. At first financial support from the Government was lacking, and, of course, his private resources were gone. As the new programs took form he struggled to continue as he might his precious research laboratory in Leningrad. He led in 1920 in founding the Mycological Society of Russia. He was one of the initiators soon after the Revolution of a High School of Phytopathology and Applied Zoology, where he lectured. In 1921 he received the support of the new government in a journey of 5 months to the United States. He visited the various phytopathological centers in Washington and the several States, making warm friendships and gaining inspiration and information for the development of his later phytopathological projects. Probably there was no happier period in the last decade of his life. During this visit to the United States he became a patron of the American Phytopathological Society. When Fawcett visited him in 1923 he reports him stimulated by the memory of his visit to this country, working personally with characteristic energy and enthusiasm in spite of lack of adequate means or assistance. As the new government gained momentum these were increasingly granted. In 1927 his scientific friends and associates honored him on the 35th jubilee of his scientific career. In recognition of his services the Government of the U. S. S. R. then applied his name to the mycological

[4] Concerning Jaczewski's national service we are permitted to quote from a personal letter recently received by the writer from Dr. N. I. Vavilov, President of the Academy of Agricultural Sciences, U. S. S. R., of which the Jaczewski Institute is a part:

"Until Dr. Jaczewski's last days he was working, dictating his book, 'Phytopathology.' The last day before his death he sent me a letter, as President of the Academy, outlining a new scheme for Phytopathological Scientific Service. It is difficult, indeed to appreciate fully the greatness of his work. He was the founder of the Phytopathological Service and the teacher of all the phytopathologists of our country. I was myself his pupil. It was in his Institute that I did my work on immunity of plants to infectious diseases."

laboratory and later elevated it in rank to a national "Institute." When the writer visited this Institut Jaczewski in 1930 he found it well housed in the former palace of the Greek Embassy, library and herbarium in excellent condition, laboratories filled with varied activities, and the center of nation-wide research and educational influence. There was a staff, including some at other stations, of perhaps 70 trained workers and an annual budget of some 400,000 rubles. It was evident, however, that he was even then reaching the limit of his bodily endurance, although not of his unyielding will to work.

Thus ended his fruitful career. Dominated to the last by worthy ideals of service through science, giving all he had in zealous pursuit of these, undaunted, undistraught, let happen what might. And, withal, as his intimate associates and family knew him, ever the same courteous, considerate gentlemen; pure-minded, open-hearted, honest seeker of right and truth, interested in art, music, and all good things; "kind and amiable to everyone," "respected, admired, and loved by friends, associates and pupils." The many friends made during his American journeys join in these tributes from his coworkers.

PATHOGENICITY AND ENVIRONAL STUDIES ON VERTICILLIUM HADROMYCOSIS

W. V. LUDBROOK[1]

(Accepted for publication May 2, 1932)

INTRODUCTION

The literature on Verticillium hadromycosis is very extensive and scattered. General surveys have been published by Van der Meer (16) in 1925 and by Rudolph (14) in 1931. The latter paper is thought to include all references worthy of note up to the early part of 1929. Wollenweber (17) and Berkeley, Madden, and Willison (2) in papers published in 1929 and 1931, respectively, give particular attention to the history of the taxonomic treatment of the vascular Verticillia in connection with reports of their own work on this aspect. As each phase of the writer's own work is taken up, a résumé of previous work on that phase will be given.

CAUSAL ORGANISMS

History of Taxonomic Treatment

Reinke and Berthold (13) were the first to isolate, describe, and name the causal organism of Verticillium hadromycosis. They apparently did not realize the possibility of taxonomic confusion arising from the variability of the pathogen. Their diagnosis of the species *V. albo-atrum* R. and B. undoubtedly includes the type forming dark hyphae but leaves some room for doubt as to whether it was intended to include also the type forming microsclerotia. Klebahn (12) isolated a vascular Verticillium forming microsclerotia, which he considered to differ from Reinke and Berthold's description of *V. albo-atrum* enough to warrant the establishment of a new species, *V. dahliae* Klebahn. This included vascular Verticillia forming microsclerotia, the older designation being reserved for types forming dark hyphae and not microsclerotia. Van der Meer (16), Dufrenoy (8), and Berkeley, Madden, and Willison (2) uphold this viewpoint, pointing out that the difference in morphology may be accompanied by a difference in

[1] The writer wishes thankfully to acknowledge his indebtedness to the following: (1) Dr. J. C. Walker, for suggesting the problem and for his advice and encouragement throughout the investigation; (2) other members of the Department of Plant Pathology, University of Wisconsin, for assistance in various ways; (3) the many workers who sent cultures of *Verticillium* spp. on request; (4) the University of Wisconsin, for granting the writer an honorary fellowship in plant pathology during the investigation, which was carried on at this institution; (5) the Council for Scientific and Industrial Research of the Australian Government, whose award of a research studentship to the writer made the investigation possible.

pathogenicity. Carpenter (6), Wollenweber (17), and Rudolph (14) consider that Reinke and Berthold's description was meant to include both types, which together with several other species of vascular Verticillia should probably be regarded as varieties or forms of *V. albo-atrum* R. and B. The papers of Rudolph (14), Wollenweber (17), and Berkeley, Madden, and Willison (2) may be consulted for more complete reviews of the literature on the question of taxonomy.

Whilst the writer has not studied the question of the validity of species of vascular Verticillia other than *Verticillium albo-atrum* and *V. dahliae*, he has no hesitation in supporting the views of Klebahn, Van der Meer, and Berkeley, Madden, and Willison on the desirability of treating these two as separate species. It was found that 45 out of 46 cultures, collected from widely different hosts and localities, could be readily and definitely classed under one or the other of the two species as defined by Klebahn. The remaining culture did not form any dark mycelium[2] whilst under the writer's observation and, therefore, could not be assigned to either of these species.

Classification of Pure Cultures Studied

The cultures of vascular Verticillia studied in the present investigation are listed in table 1. The collection comprises 46 cultures, coming from 13 States of the United States, 4 European countries, and 4 States of Australia. They were isolated from 20 different host species belonging to 17 genera. The cultures forming dark mycelium have been classified according to the views of Klebahn and Van der Meer, *i.e.*, all those forming definite microsclerotia have been placed in the species *Verticillium dahliae* Klebahn, and all those forming dark hyphae without microsclerotia have been classified as *V. albo-atrum* R. and B. Figure 1 illustrates the microscopic appearance of dark mycelium from a typical culture of *V. dahliae* and 2 types of *V. albo-atrum*. The one culture that formed no dark mycelium (No. 5 in Table 1) at first formed no verticillate conidiophores, though it formed conidia fairly readily on thin unbranched or irregularly branched conidiophores. It was not until near the end of the investigation that a few verticillate conidiophores were found in a portion of an agar plate culture that had been macerated with a scalpel. As the writer was not until then satisfied that it was a Verticillium, it was not given special attention. Its peculiarities were not altered by three consecutive plant passages. The symptoms produced in egg-plant were similar to those caused by *V. dahliae* or *V. albo-atrum*. Berkeley has used the term "*album* group" to distinguish vascular Verticillia forming no dark mycelium. In a recent letter to the writer he states that many cultures

[2] The term "dark mycelium" is used here to include both microsclerotia and dark hyphae not forming microsclerotia.

TABLE 1.—*List of pure cultures of Verticillium spp. studied, classified according to species and host*

No.	Host	Locality	No.	Host	Locality
V. albo-atrum			*V. dahliae* cont'd.		
12	Tomato	Oregon	2	Cotton	Illinois
27	"	England[a]	13	"	Tennessee
35	"	Wisconsin	45	"	Queensland
14	Potato	England	46	"	"
17	"	United States	18	African daisy	Iowa
31	"	Maine	40	Aster	Germany
38	Hop	England	41	"	"
39	Cucumber	Ohio	21	Chrysanthemum	Washington
			22	"	England
"*album*" *type*			4	Dahlia	Illinois
5	Black raspberry	Oregon	3	Lupin	"
			15	Snapdragon	United States
V. dahliae			11	Apricot	California
1	Eggplant	Wisconsin (Racine)	42	"	Victoria
20	"	" (Madison)	29	Aralia	New York
24	"	" (Racine)	32	Barberry	"
25	"	"	30	Daphne	Europe
34	"	Pennsylvania	16	Elm	United States
7	Tomato	Ohio (Toledo)	28	"	Delaware
8	"	" (Cleveland)	33	"	New York
9	"	California	23	Maple	Rhode Island
26	"	Guernsey, C. I.	10	Raspberry	California
43	"	South Australia	37	"	England
44	"	"	6	Sumac	Illinois
19	Potato	Wisconsin	48	Host unknown	New South Wales

[a] Dark hyphae longer and coarser, and darkened bases of conidiophores more conspicuous than in the other cultures of *V. albo-atrum*. (Fig. 1, C.)

thus classified by him have subsequently formed dark mycelium under special cultural conditions, *e.g.*, when grown on potato plugs. Possibly on further study culture No. 5 could be referred to *V. dahliae* or *V. albo-atrum*, but for the purposes of this paper it will be tentatively designated "*album* type."

A given isolation of the fungus often varied as to the rapidity and profusion with which dark hyphae or microsclerotia were formed under varying conditions of growth and length of time in artificial culture. This has been the usual experience of workers with this group of fungi. The writer's classification into species is based finally upon observations of cultures on potato-dextrose-agar slants, which had been kept at about 12°[3] without transfer for 12 months. The agar in these cultures had dried

[3] All temperatures are in degrees Centigrade.

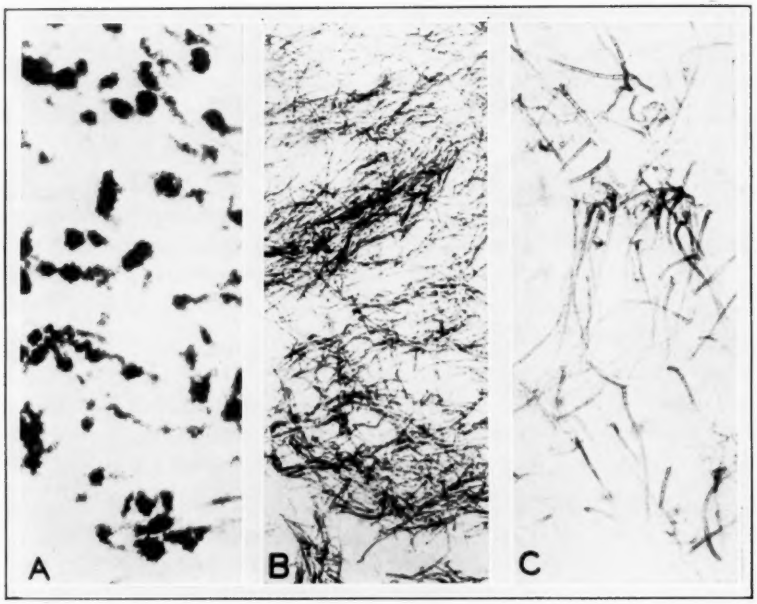

FIG. 1. A. *Verticillium dahliae*, showing typical microsclerotia with attached chains of chlamydospore-like cells. B. *V. albo-atrum*, showing typical dark hyphae. C. *V. albo-atrum*, culture No. 27 (see Table 1, footnote a). All unstained. ×60.

down to a tenth or less of its original bulk, but the cultures were all readily viable, as was proved by transfer. Several younger transfers were also observed in each case. No difficulty was experienced in making a sharp distinction between the two species in any of the cultures dealt with, except No. 5. When agar slants were inoculated with a spore suspension obtained by mixing suspensions from single-spore cultures of No. 25 (*Verticillium dahliae*) and No. 35 (*V. albo-atrum*) each species produced its characteristic dark mycelium in the resultant mixed growth. It is therefore thought that if any of the cultures had contained both types, this fact would have been evident on microscopic examination. In no case were both types found by microscopic examination in the same culture, though single-spore isolations were made in only a few instances.

At least 12 of the cultures (8 of *Verticillium dahliae* and 4 of *V. albo-atrum*) produced no dark mycelium until the transfers were several weeks old. Such cultures grown on prune-agar plates at 22° had not produced dark mycelium at the end of 3 weeks, while others produced it in 1 week or less. Cultures 1, 3, 13, and 20, under the same conditions, produced dark mycelium only in small sectors of the colonies. Since these cultures were not from single-spore isolations, the sectors may have been due to a

mixture of races. The white portions of these colonies later produced dark mycelium. The cultural variability of these 2 species, and more particularly of *V. dahliae,* is such as to suggest the possibility of hyphal fusions between different races being responsible for the variations, as has been proposed by Brierley (5) for similar variability in other fungi. In a paper entitled "The Mechanism of Variation in Imperfect Fungi; *Botrytis cinerea*" accepted for publication in Phytopathology (1932) Hansen and Smith mention *V. albo-atrum* as one of several fungi in the case of which they have evidence indicating that variation may be due to "nuclear assortment" resulting from hyphal fusions. Thus each multinucleate conidium may give rise to more than one genotype.

In spite of these variations, no culture producing microsclerotia was ever observed to produce dark hyphae (except short, rounded or irregular cells closely associated with the microsclerotia) nor was any culture producing typical dark hyphae ever observed to form microsclerotia. It was, therefore, considered expedient for the purposes of this paper to classify the collection of cultures into the two simple species *Verticillium dahliae* Klebahn and *V. albo-atrum* R. and B., with the exception of No. 5, tentatively designated "*album* type." The desirability of treating *V. dahliae* and *V. albo-atrum* as distinct species is affirmed by some authors and questioned by others, as previously mentioned. In the hope of throwing further light upon this problem, the writer obtained as representative a collection of cultures as possible in order to compare the pathogenicity and temperature relations of (1) members of the two species as separate groups and (2) different isolations of the same species.

Geographic Distribution of Species

From table 1, *Verticillium dahliae* is seen to include over 80 per cent of the cultures obtained. The numbers dealt with are, of course, much too small to warrant the drawing of definite conclusions with regard to the relative distribution of the 2 species, but it may be pointed out that all the cultures of *V. albo-atrum* came from northern United States and England, where the summers are relatively cool, while none was obtained from the warmer regions in southern United States or Australia. *Verticillium dahliae,* on the other hand, appeared to be widely distributed in both warmer and cooler regions. This agrees with the findings of previous workers and the writer, that *V. dahliae* has a higher maximum temperature than has *V. albo-atrum.*

Temperature Relations of Verticillium dahliae and V. albo-atrum in Culture

Bewley (4) stated that "the minimum, optimum, and maximum temperatures for growth in pure culture of the strains of *V. albo-atrum*

utilized for the inoculations were 4.4° C., 23.3° C., and 30° C. respectively," but he did not indicate whether the cultures used were of the *V. dahliae* or *V. albo-atrum* type. It is evident from his description of his collection of cultures that both types were included by him under the simple designation *V. albo-atrum*, as was the case with most of the earlier workers. Chaudhuri (7) gives 10°, 22.5°, and 27° as the minimum, optimum, and maximum for the culture he used, which he describes simply as *V. albo-atrum*. Edson and Shapovalov (9) and Wollenweber (17) have found the maximum temperature for certain cultures of vascular Verticillia, not producing microsclerotia, to be about 30° to 31°, while the maximum for cultures producing microsclerotia was distinctly higher, in some cases 35° to 36°. The former workers used one culture of each type, and the latter 2 asclerotial and 8 sclerotial types, all of which were classified by these authors as *V. albo-atrum* or varieties of the same.

The results of most temperature studies by previous workers agree on the point that, in general, the optimum temperature for growth of vascular Verticillia in culture is in the region of 23°, though Pötschke in a brief note gives 28° for a Verticillium causing hadromycosis in horse-radish. The maxima reported vary from 27° to 37°. Where the type of fungus used is described, cultures of the *V. albo-atrum* type generally appear to have produced little or no growth at 30°, while those of the *V. dahliae* type grew fairly well at 30° and in some cases at higher temperatures. The writer's temperature studies in culture were, therefore, concerned chiefly with the upper end of the temperature range in order to determine whether or not the higher maximum were peculiar to the *V. dahliae* group.

Experimental Data

Each culture was inoculated by mycelial transfer in 3 places on a potato-dextrose-agar plate and grown at 28°. The diameter of each colony was measured at the end of the 3rd and the 10th days after inoculation. Thus, the average total increase in diameter over a period of 7 days was calculated for each culture. The values so obtained for the 8 cultures of *Verticillium albo-atrum* averaged 5 mm. (maximum = 6, minimum = 4); for the 37 cultures of *V. dahliae*, the average was 22 mm., the maximum 28, and the minimum 14. At 30°, none of the 8 cultures of *V. albo-atrum* made any appreciable growth. Twelve cultures of *V. dahliae* were grown at 30° and gave average total increases of colony diameter ranging from 3 to 15 mm. in 7 days at 30°. The culture giving 15 mm. increase at 30° was grown at 22° and 28°, 3 colonies at each temperature, in 2 separate experiments. The increase in colony diameter averaged 30 mm. at 22° and 32 mm. at 28° on both occasions. Two typical cultures of each species grown at 22° gave increases of colony diameter ranging between 24 and 29

mm. in 7 days. With regard to the reliability of increase in colony diameter as a measure of growth, Chaudhuri (7) states that he found it reliable for any one culture grown at different temperatures under conditions identical in other respects. It probably does not give an accurate comparison of the growth rates of different cultures even under identical conditions. Therefore, the different amounts of increase in colony diameter of the different cultures of *V. dahliae* when grown at the same temperature do not necessarily indicate different amounts of actual growth, *i.e.,* production of fungal material. As regards the comparison of *V. dahliae* with *V. albo-atrum* at 28°, the difference in average increase of colony diameter is thought to be too great to admit of the possibility that it was not accompanied by a difference in actual growth rate, although the difference may not have been so large as would appear from the measurements of colony diameter. Furthermore, as previously stated, there was relatively little difference between the rates of increase of colony diameter of representatives from each species at 22°.

Thus, the writer's study of a collection of cultures more extensive than those used by previous workers shows that the upper limit for *Verticillium albo-atrum* lies between 28° and 30°, while *V. dahliae* is generally characterized by a distinctly higher maximum temperature for growth in culture. A corresponding difference in the maximum temperatures for disease production will later be pointed out. The optimum temperature for *V. albo-atrum* (in the broader sense) is usually stated in the literature as about 23°, but 1 culture of *V. dahliae* gave slightly more growth at 28° than at 22°, indicating that in some cases the optimum range of temperature extends higher than has hitherto been realized, except for the report of Pötschke mentioned above.

PATHOGENICITY STUDIES

Rudolph (14) summarized the cross inoculations recorded as having given positive results up to 1928 in a table including Verticillium cultures from at least 25 different hosts. Each of these cultures had been inoculated into one or more of 27 hosts other than that from which it was isolated. Negative results were obtained in many cases, but they cannot always be regarded as conclusive, due to the marked and imperfectly understood influence of environment on the disease. It would seem that there is little or nothing approaching strict biologic specialization amongst the vascular Verticillia, but important differences in pathogenicity between *V. dahliae* and *V. albo-atrum* have been recorded. It has been shown by Van der Meer (16) and by Berkeley, Madden, and Willison (2) that cultures of *V. albo-atrum* are often much more pathogenic than those of *V. dahliae* to certain hosts, particularly tomato, potato, and cucumber. The latter

authors produced definite disease in cucumber with each of 5 cultures of *V. albo-atrum,* but none of 32 cultures of *V. dahliae* produced any symptoms on cucumber. Seedlings of *Dahlia* sp. were affected by one culture only, a type culture of *V. dahliae* Kleb. Tomatoes were severely affected by all 5 cultures of *V. albo-atrum,* while different cultures of *V. dahliae* varied from strong to very weak parasitism on this host.

Susceptibility of Various Hosts to Verticillium Hadromycosis

The following field experiments were conducted by the writer during 1930, in which 15 plant species previously reported as susceptible to Verticillium hadromycosis were used. The aims were to compare the susceptibility of different hosts to the disease and to see whether any evidence of biologic specialization could be found. No such evidence was found. The tomato and potato, which gave negative or doubtful results in these experiments, later proved much more susceptible in greenhouse experiments, so that all the negative results obtained in these field experiments are possibly due merely to conditions unfavorable for disease development. The results are therefore thought to be of value only as an indication of the relative susceptibility of the hosts used under the conditions of the experiment, which were as far as possible uniform, except for the 2 methods of inoculation employed. The temperatures in the field were approximately as given in figure 8.[4] Inoculations were performed during the first 2 weeks in June, except those on the potato, sets of which were planted in inoculated soil during the 3rd week in May. Where external symptoms appeared, they became evident within a month of inoculation. Inoculations of herbaceous plants were made by mixing 40 gm. of corn-meal-sand inoculum with the soil into which each seed or seedling was planted. Woody plants were inoculated by introducing spore suspension or mycelium into wounds on individual shoots. The wounds were then covered with nurseryman's tape. Aseptic conditions were not maintained, but controls were run and reisolations made from diseased plants from tissue at least 5 cm. from the point of inoculation. No sign of disease was ever observed in the controls except in 6 eggplants each of which was within 3 ft. of plants heavily inoculated with corn-meal-sand inoculum. In most cases results are based on inoculations of 20 to 40 plants. Nine cultures of *V. dahliae,* isolated from 8 different hosts, and 1 culture each of *V. albo-atrum* and the *"album"* type were used. Eggplants, peppers, potatoes, tomatoes, and cucumbers were inoculated with the majority of these cultures; the remainder of the hosts were all inoculated with a culture of *V. dahliae* from eggplant.

[4] A full discussion of the data in this figure will be found under the heading, "Environal Studies—Temperature Relations in the Field."

Under the conditions of the experiment, the eggplant appeared to be the most susceptible of the hosts used; cotton, Antirrhinum, and okra were also severely affected by *Verticillium dahliae*. Individual shoots of garden rose suffered rapid withering of all the leaves above the point of wound inoculation with *V. dahliae;* milder symptoms (slow discoloration and withering of the leaves just above the wound) were produced by *V. dahliae* in shoots of wild sumac (*Rhus typhina*), wild blackberry and wild raspberry. Noninoculated wounds in control shoots caused no symptoms. Potatoes and tomatoes displayed no external symptoms of disease when inoculated with *V. albo-atrum* or *V. dahliae,* though successful reisolations were made in some cases from the xylem of the stem just above ground level. No symptoms at all were displayed by sweet pea, lupin, pepper, or cucumber[5] inoculated with *V. dahliae.*

Pathogenicity of Verticillium dahliae and V. albo-atrum. Greenhouse Experiments

Potted eggplants, tomatoes, and potatoes were inoculated with cultures of *V. dahliae* (No. 25) and *V. albo-atrum* (No. 35), in some cases through wounds at the base of the stem and in others by mixing corn-meal-sand inoculum with the soil. Ten plants of each kind were subjected to each method of treatment. Ten controls of each were grown in soil containing sterilized inoculum, and 10 were wounded at the base of the stem. The plants were kept at about 20°. In each case symptoms of disease became evident in the plants inoculated with *V. albo-atrum* a week or more earlier than in those inoculated with *V. dahliae,* and the former were much more severely diseased than the latter. The difference was less conspicuous in the eggplants, in which marked symptoms were caused by both species of Verticillium. Nevertheless, *V. albo-atrum* caused a reduction of about 40 per cent in the dry weight of the inoculated plants, as compared with about 10 per cent in the case of *V. dahliae,* after 6 weeks' growth. In the tomatoes and potatoes the only symptom caused by *V. dahliae* was a slight retardation of growth without wilting or withering of the leaves, while *V. albo-atrum* produced a much greater stunting and also wilting and death of the lower leaves of inoculated plants.

These experiments were repeated three times with eggplant and tomato and twice with potato, similar results being obtained each time. They were also repeated with cultures 14 (*Verticillium albo-atrum*) and 44 (*V. dahliae*), which showed a like difference in pathogenicity. As will be described under the heading "Environal Studies," culture No. 25 (*V. dahliae*) failed to produce definite symptoms of disease in tomato plants at

[5] Successful inoculations of cucumber with *V. albo-atrum* were later performed in the greenhouse at 20°. *Verticillium dahliae* produced no effect.

soil temperatures between 16° and 30°, while No. 35 (*V. albo-atrum*) produced marked symptoms from 16° to 26° (Fig. 7). The plants in this soil-temperature-tank experiment made much more vigorous and rank growth than did the potted plants described above.

In several further series of inoculations upon potted tomato plants at mean temperatures between 18° and 23°, all the available cultures were tested for pathogenicity. The plants were grown for 4 to 6 weeks after inoculation, which was found to be ample time for the development of marked symptoms in plants inoculated with pathogenic cultures. Six, 7, or in most cases 9 plants were inoculated with each culture in each series. Inoculations were performed in most cases by pouring an aqueous suspension of a macerated potato-dextrose-agar culture of the fungus around the roots when potting the seedlings. In 2 series, wound-inoculations into the stems were made, and, in another, corn-meal-sand inoculum was mixed with the soil. The severity of the symptoms and the average dry weight of stems and leaves were recorded for the plants inoculated with each culture in each series of inoculations. Not all of the cultures were tested in more than one series of inoculations, but representative cultures of each species were tested in from 2 to 5 different series and gave consistent results. A standard culture of each species was included in every series.

TABLE 2.—*Summary of results of pathogenicity experiments with potted tomato plants. The weights of inoculated plants given are relative to those of the noninoculated plants, which were taken as 100 in each series.*

Species of fungus	Symptoms moderate to severe			Symptoms slight or absent		
	Number of cultures	Total number of trials[a]	Average dry weight of tops	Number of cultures	Total number of trials[a]	Average dry weight of tops
Verticillium dahliae	9	16	78	28	35	107
V. albo-atrum	6	14	59	2	6	98
"*album*" type				1	4	90

[a] This number is obtained by writing down for each culture the number of separate series of tests in which that culture was included (varying from 1 to 5 for different cultures) and totalling these figures.

The results are summarized in table 2. Six out of 8 cultures of *Verticillium albo-atrum* were severely pathogenic to tomato, causing conspicuous mottling with yellow, wilting and withering of the lower leaves, also marked reduction in the dry weights of stems and leaves as compared

with those of noninoculated plants, the latter being taken as 100 in each series. The other 2 cultures of *V. albo-atrum* (Nos. 17 and 38) displayed little or no pathogenicity. Nine out of 37 cultures of *V. dahliae* produced marked to severe disease symptoms and reductions in weight, though on the average less severe than those due to *V. albo-atrum*. The remaining 28 cultures of *V. dahliae* and the one culture of the *album* type displayed little or no pathogenicity. Two single-spore cultures of *V. dahliae* that were strongly pathogenic to the rather weak plants used in one series proved to be relatively less pathogenic to the more vigorous plants used in the remainder of the experiments, as compared with the typical *V. albo-atrum* cultures. Thus, the relative severity of disease produced by a given culture may vary according to the general vigor of the host plants. The above results agree with those of previous workers except that 2 cultures of *V. albo-atrum* were found to exhibit little pathogenicity to tomato. These cultures were somewhat atypical in cultural characters, and one had been in culture for an unknown length of time. However, it is not considered that variation in the age of the cultures used seriously vitiates these results. Most of them were isolated during the latter half of 1930. Culture No. 31 (*V. albo-atrum*) had been grown on artificial media continuously since 1924, and No. 7 (*V. dahliae*) since 1927. Each was one of the most pathogenic of its own species.

Field Experiments

Field experiments were conducted in 1930 and 1931 to investigate the possibility of differences in pathogenicity between different cultures of *V. albo-atrum* and *V. dahliae*. In 1930, the hosts used were eggplant, tomato, potato, cucumber, and pepper. One culture of *V. albo-atrum* and 8 of *V. dahliae* were used, 50 gm. of corn-meal-sand inoculum being mixed with the soil in the bottom of the hole into which each seedling was transplanted. Twenty plants of each host were inoculated with each culture. Ten noninoculated plants of each host grown between each pair of inoculated strips made good growth and were free of disease. The temperatures during the growing periods of 1930 and 1931 are shown in figure 8.

Of the inoculated plants in 1930, the potatoes, cucumbers, and peppers showed no signs of disease, whatever. The tomatoes were unaffected except for slight vascular discoloration at the base of the stem produced by 6 out of 9 cultures, including *Verticillium albo-atrum*. At the end of the growing period but before the plants had begun to die off, the fungus was reisolated from 13 out of 30 such tomato plants from the stem at least an inch above ground level. Nearly all the eggplants inoculated were stunted to about ⅔ the size of the noninoculated plants; their lower leaves became mottled with yellow, wilted, and dried up. There was no appreciable dif-

ference in severity of disease between the plants inoculated with the different cultures, though the cultures all showed marked differences in appearance when grown on agar plates.

In 1931 similar experiments were carried out in which 4 cultures of *Verticillium albo-atrum* and 3 of *V. dahliae* were used. The same general procedure and hosts were used, except that peppers were omitted and the quantities of inoculum were varied between 20 and 80 gm. per plant without visibly influencing the severity of the disease. In recording the results, an arbitrary scale of severity was adopted, ranging from 0 (plant killed without making a significant amount of growth after inoculation) to 5 (plant unaffected). The eggplants showed much less disease than in 1930. Towards the end of the growing period, each inoculated eggplant was assigned one of the figures of the above scale. The average values thus obtained for the lots inoculated with *V. albo-atrum* cultures were 4.5 (culture No. 39), 3 (No. 14), 3.5 (No. 35), and 4 (No. 27) and for those inoculated with *V. dahliae* cultures, 5 (No. 44), 3.5 (No. 25), and 4.3 (No. 21). The tomatoes were unaffected, except for slight vascular discoloration at the base of the stem in about a half of the plants inoculated with culture No. 14 (*V. albo-atrum*). In the potatoes, one culture of each species (Nos. 21 and 35) produced slight vascular discoloration in the base of the stem; the remainder had no effect.

These results cannot be held to afford satisfactory evidence for or against differences in pathogenicity, in view of the small differences and amounts of disease observed. The experiments are described mainly because they indicate that symptoms of Verticillium hadromycosis may partially or wholly fail to develop in artificially inoculated field-grown eggplants, tomatoes, and potatoes under mean-temperature conditions found favorable for disease of these hosts when grown in soil-temperature tanks. These experiments will be further discussed from the environal viewpoint after describing the soil-temperature tank experiments.

ENVIRONAL STUDIES

Literature

Temperature appears to be the most important environal factor influencing the severity of this disease (Rudolph, 14). Studies of temperature effect on growth of the fungus in culture have already been considered, and attention will here be confined to observations on the effect of temperature on the occurrence and severity of the disease, treating firstly of greenhouse and secondly of field studies. The effect of soil moisture also will be considered in connection with the greenhouse studies on eggplant.

Bewley (3, 4) made observations in commercial glass houses, which indicated that temperatures between 15.5° and 24° were favorable for

the disease in tomato, the optimum being about 22°. The disease was partially counteracted by shading. Schoevers (15) gives 25° as approximately the maximum temperature at which the disease is likely to be evident in tomatoes. Alexander (1) gives a brief preliminary report of soil-temperature-tank experiments with tomatoes. Sterilized soil was inoculated with *Verticillium albo-atrum*. Severe disease occurred at soil temperatures between 12° and 24°. At 28° plants showed only slight symptoms and made as good growth as the noninoculated plants.

Soil moisture is recorded in the last-mentioned report as having no influence on the disease. Many opinions based on observational evidence of moisture relations have been published. Extremes of both high and low moisture have been reported as aggravating the disease in various hosts. Bewley (3) considers that heavy watering increases the severity of the disease in tomatoes. Van der Meer (16) reports as follows: "Some pot experiments with the potato variety 'Eigenheimer' inoculated at the stem base with *V. albo-atrum* or *V. dahliae* (potato) were arranged as follows. One set of plants was kept moist under glass, a second was watered every day, and a third was placed under a penthouse and kept as dry as possible. It appeared that the development of the disease was much more rapid in drought, and that under very moist conditions the plants suffered but little from the disease."

Temperature and Moisture Relations Under Controlled Conditions

Methods. Standard Wisconsin soil-temperature tanks were used, the soil temperature being controlled to within half a degree Centigrade. The air temperature was maintained at or above a constant minimum, variations being recorded by a thermograph. Mean daily and weekly air temperatures were determined by averaging the thermograph readings at intervals of 2 hours throughout the period in question. Soil moistures were maintained as constant as possible by periodical weighing and addition of the required amount of water. At the end of the first experiment with eggplant a test of representative soil samples indicated that the average moisture content of each can had not changed by more than about 2.5 per cent of the moisture-holding capacity from the desired moisture content, though there was a tendency for the water to accumulate at the bottom of the can in the case of 85 or 95 per cent saturated soil. Except where the effect of higher or lower soil moistures was being investigated, the soil was kept 65 per cent saturated with water.

The cultures used were as follows, except where otherwise stated. The *Verticillium dahliae* culture chiefly used (No. 25 in Table 1) was from an eggplant at Racine, Wisconsin; the *V. albo-atrum* culture chiefly used (No. 35 in Table 1) was from a tomato plant in a greenhouse near Milwaukee, Wisconsin. Both were isolated by the writer in November, 1930. In each

case, 1 of a number of single-spore isolations was chosen and used throughout this series of experiments, except that cultures from other sources were used for comparison in certain specified cases. The fungus was grown on corn-meal sand for about 10 days: about 50 gm. for eggplants and 80 to 160 gm. for potatoes and tomatoes were mixed with the top third of the soil in each inoculated can. A like amount of sterilized inoculum was mixed with the soil in each control can. Nonsterilized soil was used throughout all the tank experiments to be described, and no sign of disease was found in any noninoculated plant.

The varieties of host plants used were Black Beauty eggplant, Bonny Best tomato, and Bliss Triumph potato. Three seedlings about 3 in. high were planted in each can in the case of eggplant and tomato; 2 pieces of potato tuber were planted, only 2 shoots per can being allowed to develop. The cans were 6 by 10 in. The time of first appearance of definite symptoms (paleness and flaccidity of the distal or sometimes lateral margins of the lower leaves) was noted for each can. All the plants in each can usually showed symptoms within a day or two of one another, except in the case of the potatoes, where the time for each plant was recorded. Unless otherwise stated, the experiments were continued until the nondiseased plants were about to become too large for healthy growth in the cans. In order to obtain a roughly quantitative expression of the results, the plants in each can were then severed from the roots at ground level, dried for 24 hours at 100° C., and weighed. The weights per can of noninoculated and inoculated plants were tabulated for each experiment. The reductions in the weights of the inoculated plants were taken as roughly indicative of the relative severity of the disease and were expressed as percentages of the weights of the corresponding noninoculated plants, to give a uniform basis for comparison. It must be emphasized that these weights and percentages are in every case liable to a high experimental error, due to the small numbers of plants used. They are employed merely to enable the results of these particular experiments to be stated more explicitly than would otherwise be possible.

EGGPLANT HADROMYCOSIS

Soil temperature and moisture relations at 19° to 22° air temperature. The 1st experiment was conducted during the 7 weeks beginning February 7, 1931, and the 2nd during the 6 weeks beginning April 9, 1931. The mean air temperature was 19° during the first period and 22° during the second. The soil temperatures and moistures used are enumerated in table 3,[6] together with the weights of noninoculated plants and percentages

[6] In addition to these, a soil temperature of 12° and a soil moisture of 35 per cent of saturation were tried in the first experiment. Severe disease occurred under these conditions, but the uninoculated plants made so little growth that the results will not be further considered.

TABLE 3.—*Influence of soil temperature and moisture on (1) dry weights[a] of noninoculated eggplants, (2) reductions[b] in weights of eggplants inoculated with Verticillium dahliae in 1st and 2nd tank experiments*

Soil moisture in percentage of moisture-holding capacity	Experiment No.	Soil temperatures (mean air temperature = 19° for 1st and 22° for 2nd experiment)											
		16°		20°		24°		28°		30°		32°[c]	
		Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants	Weight of noninoc. plants	Percentage reduction of inoc. plants
45	1	2.2[a]	70[b]	6.3	86	8.6	85	5.6	80			5.3	26[c]
	2					6.0	80	5.4	72	5.6	43	5.1	−6[b]
65	1	2.4	54	8.5	82	9.7	74	12.7	80			10.6	20
	2					11.2	64	12.3	64	11.9	68	10.0	17
85	1	1.7	29	7.1	73	11.0	75	14.5	61			13.0	21
	2					13.0	65	14.7	37	21.7	47	16.0	−2
95	2					16.3	73	19.7	49				

[a] Each weight given is the total weight in grams of the 3 plants in 1 can (excluding roots) at the end of 7 weeks' (1st experiment) or of 6 weeks' (2nd experiment) growth.

[b] The amounts by which the dry weights of the inoculated plants fell short of those of the corresponding noninoculated plants are expressed as percentages of the latter. A negative percentage, therefore, signifies that the inoculated plants weighed more than the noninoculated plants.

[c] There were no symptoms of disease at 32°, and the reductions in weight that occurred in some cases are thought to be attributable to experimental error.

of reduction in the weights of inoculated plants. *Verticillium dahliae* (No. 25) was used in both experiments. Space permitted the inclusion of only one inoculated and one noninoculated can in each experiment for each set of conditions, but the results of the 2nd experiment satisfactorily confirmed those of the 1st with regard to temperature, and subsequent comparative experiments with other cultures of Verticillium gave abundant confirmation of results at the critical temperatures.

The incubation periods in the 1st experiment varied from 9 to 16 days and in the 2nd from 16 to 26 days, the increase in the 2nd experiment probably being due to the greater vigor of the plants. While the data on

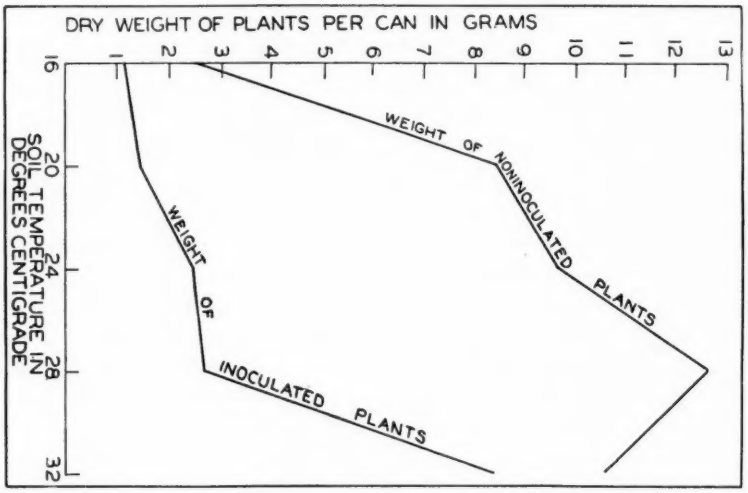

FIG. 2. Influence of soil temperature on weight of noninoculated eggplants and of those inoculated with *Verticillium dahliae* in the 1st tank experiment. Mean air temperature, 19°, soil moisture, 65 per cent of the moisture-holding capacity.

this point are not very consistent, they indicate that the appearance of symptoms may be delayed a few days by very high (28° or above) and by very low (16° or below) soil temperatures. The only appreciable effect of soil moisture on length of incubation period was a slight delay in the appearance of symptoms in plants grown in very moist soil at 28°. This effect was not evident above or below this temperature. It is shown graphically in figure 3.

The results of the first experiment relating to the effect of temperature on growth of inoculated and noninoculated plants in 65 per cent saturated soil are summarized graphically in figure 2. In figure 3 the data from Experiment 2 relating to the effect of 4 different soil moistures at 28° soil

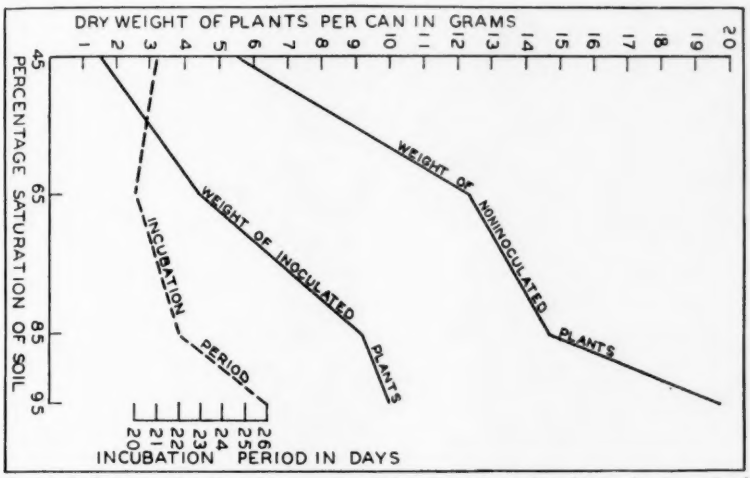
DRY WEIGHT OF PLANTS PER CAN IN GRAMS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PERCENTAGE SATURATION OF SOIL
45 65 85 95
WEIGHT OF NONINOCULATED PLANTS
WEIGHT OF INOCULATED PLANTS
INCUBATION PERIOD
20 21 22 23 24 25 26
INCUBATION PERIOD IN DAYS

FIG. 3. Influence of soil moisture on weight of noninoculated eggplants and of those inoculated with *Verticillium dahliae* and on the incubation period, in the 2nd tank experiment. Soil temperature, 28°, mean air temperature, 22°.

temperature are plotted. Table 3 contains the weights of all noninoculated plants, and the percentages of reduction in weight of all inoculated plants grown in Experiments 1 and 2. The results of all tank experiments with eggplant will be summarized at the end of the section on eggplant hadromycosis. Thirty degrees was the highest soil temperature at which definite symptoms of eggplant hadromycosis appeared; *Verticillium dahliae* was reisolated from eggplant stems 2 in. above ground level in all 3 inoculated cans at 30° soil temperature in Experiment 2. Photographs were taken of the plants in the cans in Experiment 1 after 5 weeks' growth. The influence of varying soil temperature in soil 85 per cent saturated with water is shown in figure 4 and that of varying soil moisture at a soil-temperature of 28° in figure 5, the inoculated plants being contrasted with the corresponding noninoculated plants in each case.

In order to compare *Verticillium albo-atrum* with *V. dahliae* as to the effect of soil temperature on pathogenicity, the following experiments were conducted. On September 26, 1931, 2 cans with *V. dahliae* (No. 25) and 2 with *V. albo-atrum* (No. 35) were planted with eggplants. One can inoculated with each species was kept at 28° soil temperature and the other at 30°. Two noninoculated cans were kept at each soil temperature. The mean air temperature was 20°. Definite symptoms of wilt appeared about 19 days after planting in each can inoculated with *Verticillium dahliae.* No symptoms appeared in the cans inoculated with *V. albo-atrum* until about the 35th day, and then only in the can at a soil temperature of 28°. Forty days after planting, all the tops were severed from the roots, dried and

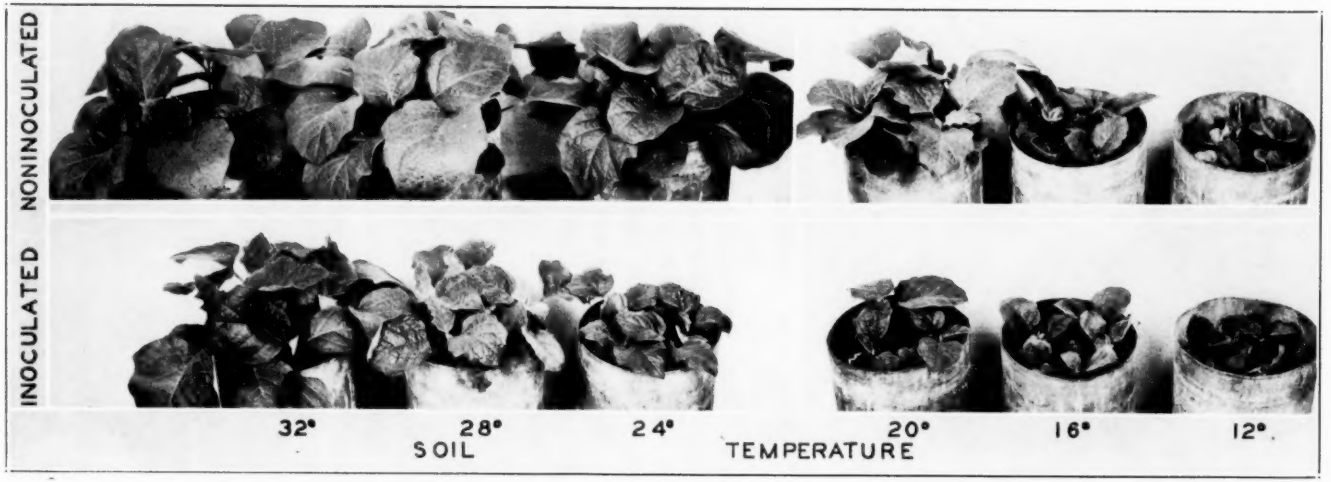

FIG. 4. Influence of soil temperature on growth of noninoculated eggplants and on severity of disease in eggplants inoculated with *Verticillium dahliae*. Soil moisture, 85 per cent of the moisture-holding capacity, mean air temperature, 19°. Five weeks after inoculation.

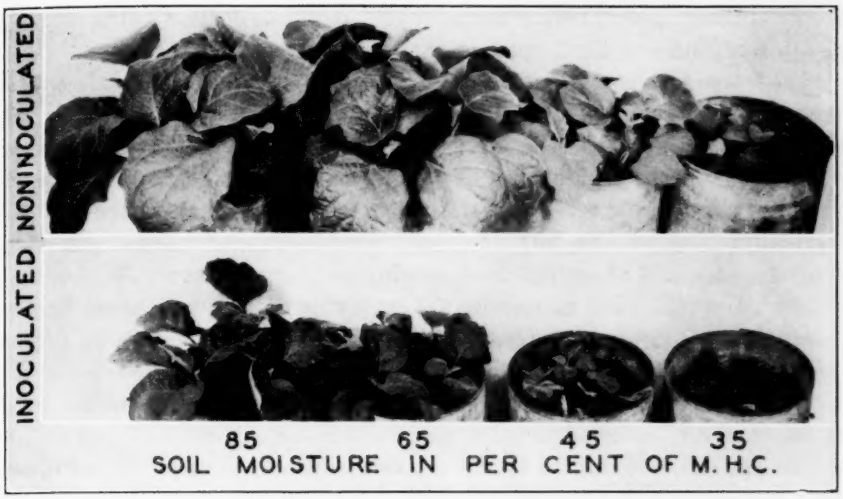

FIG. 5. Influence of soil moisture on growth of noninoculated eggplants and on severity of disease in eggplants inoculated with *Verticillium dahliae* at a soil temperature of 28° and an average air temperature of 19°. Five weeks after inoculation.

weighed. There was about a 30 per cent reduction in the weights of the plants inoculated with *V. dahliae* at 28° and 30°, with marked symptoms of disease in each case. The plants inoculated with *V. albo-atrum* were reduced in weight by 20 per cent at 28° and by 7 per cent at 30°. They displayed slight disease symptoms at 28° and none at 30°.

The same cans of soil were replanted with eggplant seedlings, and similar results again obtained. The can inoculated with *Verticillium albo-atrum* and kept at 30° soil temperature was held for 10 weeks without the appearance of any sign of disease. The experiment was repeated a third time during January and February in a new lot of freshly inoculated soil, the same cultures of Verticillium being used; one other culture of each species, Nos. 14 and 44, was also tried. The results agreed with those of the previous experiments.

A further series was run including 6 other cultures of *Verticillium albo-atrum* at soil temperatures of 28° and 30°, and 4 other cultures of *V. dahliae* at soil temperatures of 30° and 32°. The mean air temperature was about 23°. Of the *V. albo-atrum* cultures at 28°, 4 produced slight symptoms and 2 had no effect; at 30°, none had any effect. All the *V. dahliae* cultures produced definite signs of disease at 30°, but none at 32°. It is therefore concluded that whilst the maximum soil temperature at which *V. albo-atrum* can cause disease in eggplant is usually about 28° or below, it is commonly about 30° in the case of *V. dahliae*. This agrees with the dif-

ference in maximum temperature that has been demonstrated for the growth in culture of the 2 species.

Soil temperature relations at 28° to 31° air temperature. This experiment was begun on April 12, 1931, in a small glass house maintained at a minimum air temperature of 28°. On sunny days the air temperature became very high, often reaching 37° in the middle of the day and raising the mean air temperature over the 10 weeks of the experiment to 31°. *Verticillium dahliae* (No. 25) was used. One inoculated and one noninoculated can were held at each of the following soil temperatures: 22°, 24°, 26°, 28°, 30°, and 32°. The noninoculated eggplants did not make such healthy growth as those in the larger house, where the 1st and 2nd experiments were conducted. Both inoculated and noninoculated plants tended towards a spindling habit of growth with chlorotic areas between the veins of the leaves.

The periods that elapsed before the first appearance of visible symptoms were distinctly longer than in the first 2 experiments, *viz.* 29 days at 22° soil temperature and 31 days at 24°, 26°, and 28° soil temperature. No definite symptoms appeared at 30° or 32°. The lowest 3 or 4 leaves of each inoculated plant at the lowest 4 soil temperatures were wilted and withered by the disease, which progressed slowly during the next 2 or 3 weeks after the first appearance of symptoms.

The eggplants began to outgrow the disease 7 or 8 weeks after planting, presumably owing to the increasingly high air temperatures during the daytime, although the new growth in both control and inoculated plants was of the unhealthy type described above. On June 27, about 10 weeks after planting, the experiment was discontinued. The diseased leaves by that time had dropped off and the remaining foliage was free from any definite symptoms of hadromycosis at all soil temperatures. In the 22° and 24° tanks, the inoculated plants were slightly smaller than the noninoculated plants, but in the others no difference was visible. The inoculated plants at soil temperatures from 22° to 28° showed distinct brown discoloration of the vascular bundles of the stem up to 2 or 3 in. above the soil level, but those at 30° and 32° did not show any such discoloration. *Verticillium dahliae* was reisolated from the stem (about 1 in. above ground level) of the plants at 26° and 28° soil temperatures, but those at 30° failed to give any growth of the fungus on plating.

This experiment indicates that at a high air temperature (averaging about 31°) the maximum soil temperature at which *Verticillium dahliae* may cause disease symptoms in eggplant is somewhat lower than at low air temperatures. Thus, in the experiment just described, the maximum soil temperature at which disease symptoms developed was 28°, as compared with 30° in the 2nd tank experiment. Similar indications were obtained from the 1st tank experiment with tomatoes, described below.

Air temperature relations. In the experiments described above, the air temperature in most cases differed from the soil temperature. For comparison with the results of such experiments, eggplants growing in pots were inoculated through stem wounds above ground level and kept in small glass houses in which the minimum air temperatures were maintained at 17°, 20°, 24°, and 28°. Ten inoculated and 5 noninoculated plants were kept in each house. The plants were cut off at ground level 35 days after inoculation, dried, and weighed. The weights of the inoculated plants averaged about ½ those of the controls at all 4 temperatures. A 2nd experiment was performed in the 28° house only, using 10 controls and 6 plants inoculated with each of the following cultures: 14 and 35 (*Verticillium albo-atrum*), 25 and 44 (*V. dahliae*). The results agreed with those of the 1st experiment, except that the symptoms produced by the 2 cultures of *V. albo-atrum* at 28° were a little slower in appearing and less marked than those due to *V. dahliae*.

Summary. Noninoculated eggplants grew best at soil temperatures of 28° and 30°, the air temperature being 19° to 22°. The higher soil temperature was more favorable at higher soil moistures. A mean air temperature of 31°, often going up to 37° for short periods, proved less favorable than the lower air temperatures. Eggplants in pots grew better at 28° than at lower air temperatures.

Marked disease symptoms appeared, and the growth of eggplants inoculated with *Verticillium dahliae* was reduced 50 to 80 per cent as compared with that of the noninoculated plants, at soil temperatures of 16°, 20°, 24°, 28°, and 30°, the air temperatures being 19° to 22°. The reduction in growth of inoculated plants was most marked at 20°, gradually decreasing at higher temperatures up to 30°. There was in some cases a slight reduction in average weight of inoculated plants at 32°, but this was probably due to experimental error rather than to parasitism by the fungus, as there were no other symptoms and in 2 out of 6 cases the plants in inoculated soil weighed more than did the controls. Apparently the disease was slightly less severe at 16° than at 20°. At an air temperature averaging 31°, no symptoms appeared above 28° soil temperature: the symptoms at 28° and below were much less marked than at the lower air temperatures and disappeared as the mean air temperature became higher towards the close of the experiment. The maximum soil temperature for disease in plants inoculated with *V. albo-atrum* was 28° as compared with 30° for *V. dahliae*, the air temperature being 20° to 23° in each case. Potted plants inoculated with *V. dahliae* through stem wounds above soil level became diseased with approximately equal severity at air temperatures of 17°, 20°, 24°, and 28°.

Increase of soil moisture up to 95 per cent of saturation was accompanied by increased growth of noninoculated eggplants at 24° and 28°

soil temperature; at 30° and 32°, increase of soil moisture up to 85 per cent (the maximum tried at these temperatures) gave increased growth of the noninoculated plants. At 20° and 16°, noninoculated plants in soil 85 per cent saturated grew less than did those in soil 65 per cent saturated.

Soil moisture exerted no appreciable effect on severity of disease except at 28° soil temperature (air temperature 19° to 22°), where the disease was a little later in appearing and less severe in soils 85 and 95 per cent saturated than in those 45 and 65 per cent saturated with water. Probably this was merely an effect of the increased vigor of the host plants under these conditions.

The results as to temperature relations will be compared with data from field observations in the section on "Temperature Relations in the Field."

TOMATO HADROMYCOSIS

Soil temperature relations at 25° to 30° air temperature. The 1st tank experiment with tomato as host was begun on April 12, 1931. The temperatures used were determined by the space left over after requirements had been satisfied for the concurrent eggplant experiments rather than selected with suitability as the first consideration. Larger quantities of inoculum were used than in the experiments with eggplant, as some difficulty had been experienced previously in producing disease symptoms in the tomato. One-hundred-fifty grams were used in each can, made up of equal parts of corn-meal sand inoculated with the following cultures: *Verticillium dahliae* (No. 25) and *V. albo-atrum* (No. 35). One inoculated and one noninoculated can were kept during the whole experiment in the large greenhouse used for the 1st and 2nd eggplant experiments, where the air temperature averaged about 24.5° during this time (23° during the first 5 weeks). The remainder were kept for the first 5 weeks of their growth in the small greenhouse used for the eggplant experiment in which the air temperature averaged about 30° during this time. Here it became so hot during the day that both inoculated and noninoculated plants made very spindly, small-leaved, unhealthy growth, and no definite symptoms of hadromycosis developed. As soon as space became available, these cans were transferred to a larger greenhouse, where the air temperature during the rest of the experiment averaged 26°, and were here kept at lower soil temperatures, as stated in table 4, where the conditions of growth and development of disease in each inoculated can of this experiment are summarized.

It would appear from the results of this experiment that high air temperatures (including temperatures too high for the healthy growth of the host) may hinder the appearance of symptoms in plants growing in soil at temperatures suitable for the optimum development of the fungus.

Soil temperature relations at 20° air temperature. The 2nd tank ex-

TABLE 4.—*Conditions of growth and development of Verticillium hadromycosis in 1st tank experiment with tomato*

Conditions of growth				Approximate incubation period[a]	Final results	
First 5 weeks		Second 5 weeks				
Soil temperature	Average air temperature	Soil temperature	Average air temperature		Browning of vascular bundles	External symptoms
Degrees	*Degrees*	*Degrees*	*Degrees*	*Days*		
22	30	14	26		Slight at base of stem	Indefinite; stunting, drying up of lower leaves
24	"	16	"		"	"
26	"	18	"		"	"
20	23	20	"	18	Marked, visible 9 in. up stem	Marked wilting, yellowing, and withering of lower leaves
28	30	20	"		Slight at base of stem	Indefinite
24	23	24	"	18	Marked	Marked
30	30	24	"		None	Symptoms less marked; gradual drying up of lower leaves
32	"	26	"	No symptoms	None	None

[a] Where no data are given, it was not found possible to assign even an approximate date for the first appearance of symptoms.

periment with tomato was begun on September 26, 1931. The mean air temperature was 20°. Each inoculated can received a total of 150 gm. of inoculum, 50 gm. each of corn-meal-sand medium inoculated with cultures 21 and 25 (*Verticillium dahliae*) and culture 35 (*V. albo-atrum*). Two inoculated and 2 noninoculated cans were kept at each of the following soil temperatures: 20°, 22°, 24°, 26°, 28°, and 30°. It was desired to run tanks at 16° and 18°, but the lowest soil temperature obtainable at the

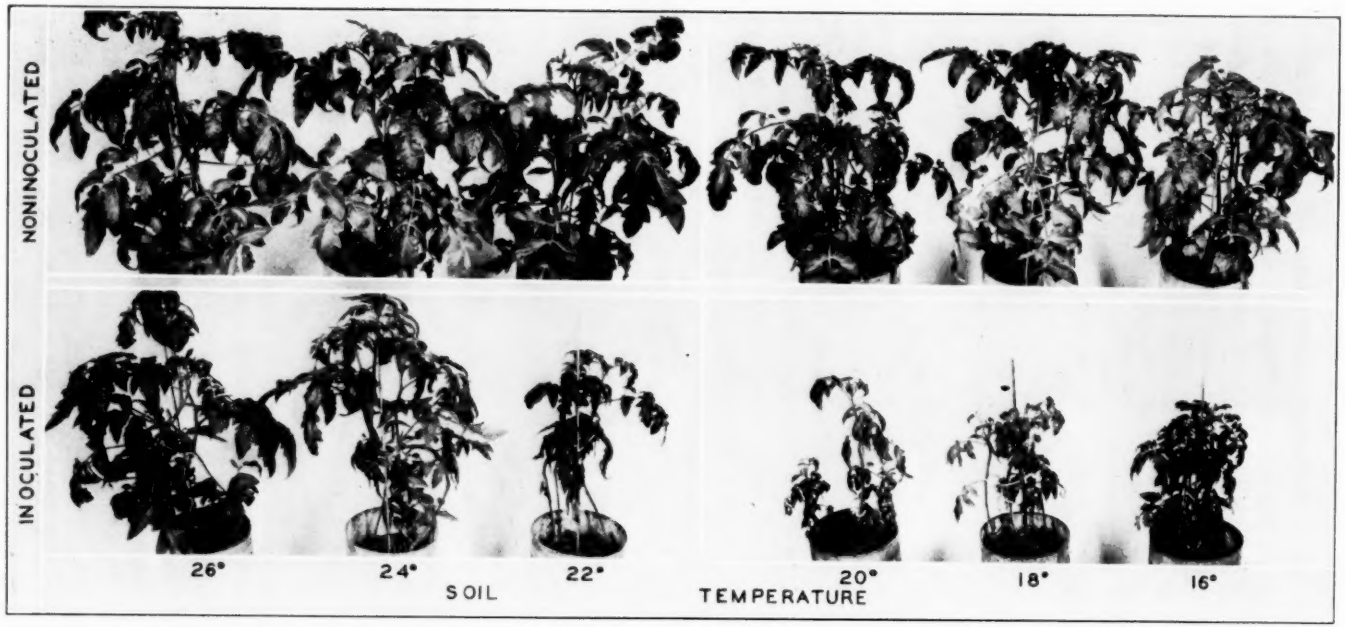

FIG. 6. Influence of soil temperature on growth of noninoculated tomatoes and on severity of disease in tomatoes inoculated with a mixture of *Verticillium albo-atrum* and *V. dahliae*. Mean air temperature, 20°, soil moisture, 65 per cent of the moisture-holding capacity. Six weeks after inoculation.

beginning of the experiment was 19°. Two tanks (with 2 inoculated and 2 noninoculated cans in each) were begun at this temperature; 18° was attained by the end of the 3rd week, and one tank was kept at this temperature. The other reached 16° by the end of the 4th week.

The incubation period in the 2nd experiment varied from 17 days at 20° soil temperature to 20 days at 22° and 25 days at 24°. Severe disease occurred at 22° and below, less severe at 24°, and still less at 26°, at which temperature no definite time could be assigned for the first appearance of symptoms. No symptoms appeared at 28° or 30°. After 6 weeks' growth the plants were photographed (Fig. 6), dried, and weighed. The weights of noninoculated plants and percentages of reduction in weights of inoculated plants are given in table 5. The noninoculated plants grew about equally well at soil temperatures from 20° to 30°.

The 3rd tank experiment with tomato was begun on February 13, 1932. It was a replication of the 2nd except that 3 cans in each tank were inoculated with a pure culture of *Verticillium albo-atrum* (No. 35) and 3 with a

TABLE 5.—*Influence of soil temperature on dry weights in grams of stems and leaves of noninoculated tomato plants from the 2nd and 3rd tank experiments and on reductions in weight of inoculated plants (expressed as percentages of the dry weights of the corresponding noninoculated plants). The results from duplicate cans are averaged in Experiment 2, and those from duplicate noninoculated and triplicate inoculated cans are averaged in Experiment 3. Each weight represents the average total dry weight of stems and leaves per can of 3 plants. Mean air temperature = 20° in each case.*

Soil temperature	Experiment 2. Inoculated with a mixture of *Verticillium albo-atrum and V. dahliae*		Experiment 3		
	Weight of noninoculated plants	Percentage of reduction in weight of inoculated plants	Weight of noninoculated plants	Percentage of reduction in weight of plants inoculated with:	
				V. albo-atrum	*V. dahliae*
Degrees					
16	9.7	72	7.1	66	−13[a]
18	10.2	59	–	–	–
20	10.4	77	11.7	68	−5[a]
22	9.1	50	11.4	62	−16[a]
24	10.8	32	12.7	68	−6[a]
26	10.9	38	12.7	53	6
28	10.0	−10[a]	12.1	6	4
30	11.1	0	10.4	−2[a]	−2[a]

[a] Where a minus sign precedes the percentage, it indicates that the weight of the inoculated plants exceeded that of the corresponding noninoculated plants by this percentage, so that the reduction in weight was a minus quantity.

pure culture of *V. dahliae* (No. 25). The remaining 2 cans were noninoculated. Of the 3 cans inoculated with each species of Verticillium at each soil temperature, 2 received 80 gm. and one 160 gm. of corn-meal-sand inoculum. The noninoculated cans each received 80 gm. of sterilized inoculum. The mean air temperature was again 20°.

In the tomato plants inoculated with *Verticillium albo-atrum* in the 3rd experiment, symptoms appeared at all soil temperatures up to and including 26° within 13 to 15 days. During the first 5 weeks' growth, no definite symptoms appeared in any of the plants inoculated with *V. dahliae*. Those inoculated with *V. dahliae* at all soil temperatures and those with *V. albo-atrum* at 28° and 30° were indistinguishable from the noninoculated plants. At the end of 5 weeks' growth the plants were dried and weighed, excepting one plant in each can at 22°, 26°, and 28°. These were kept growing at the same temperatures to see if disease would appear later in the plants not then showing it. *Verticillium albo-atrum* was isolated from the xylem in the base of the stem one in. above ground level in 3 plants at 28°, which showed slight vascular discoloration. This, like similar reisolations made from field-grown inoculated plants (see "Susceptibility of Various Hosts to Verticillium Hadromycosis") proves that the fungus may be present in the lower portions of a tomato plant without appreciably affecting its growth. At this time slight yellowing and wilting of the leaves were beginning to appear at the bases of the plants inoculated with *V. albo-atrum* at 28° and of those inoculated with *V. dahliae* at 28° and below. These plants were otherwise similar to the noninoculated plants (Fig. 7). After

FIG. 7. Pathogenicity and soil temperature relations of *Verticillium dahliae* and *V. albo-atrum* in tomato. A, noninoculated, B, inoculated with *V. dahliae*, C, inoculated with *V. albo-atrum*, all at 26° soil temperature. D, inoculated with *V. albo-atrum* at 28° soil temperature. Mean air temperature, 20°, soil moisture, 65 per cent of the moisture-holding capacity. Five weeks after inoculation.

2 more weeks these symptoms had not become any more marked, though *V. dahliae* was reisolated from the petiole of one of the lower leaves of 2

plants at 26° and 2 at 28°, which showed slight yellowing and tip withering of the lowest 2 or 3 leaves. Thus, *V. dahliae,* though present in the lower parts of the plants, did not significantly affect their growth. Three of the plants inoculated with *V. dahliae* and grown at 22° were kept for a total period of 8 weeks without further symptoms developing than those already described. The weights of plants and percentages of reduction in weight of inoculated plants at the end of 5 weeks' growth are given in table 5. In most cases the plants inoculated with *V. dahliae* weighed slightly more than the noninoculated plants. It is doubtful whether this fact has any significance. As *V. dahliae* alone did not cause appreciable amounts of disease in the 3rd experiment, it is presumed that *V. albo-atrum* was mainly responsible for the disease in both experiments.

The disease occurred with approximately equal severity in tomato plants at soil temperatures ranging from 16° to 22° in both experiments. It was a little slower in appearing and less severe at 24° and 26° in the 2nd experiment; in the 3rd it was less severe at 26° but as severe at 24° as at lower temperatures. There was no significant amount of disease at 28° or 30° in either experiment. There was no difference between the plants receiving 80 gm. of inoculum per can and those receiving 160 gm. in the 3rd experiment. Thus, the maximum soil temperature for the occurrence of Verticillium hadromycosis in tomatoes inoculated with either a mixture of both species or *Verticillium albo-atrum* alone appears to be about 26°, as compared with a maximum of 30° for eggplants inoculated with *V. dahliae* or 28° for eggplants inoculated with *V. albo-atrum,* the air temperature in each case averaging 20°.

Potato Hadromycosis. This experiment was run in the same tanks as were used for the 2nd tomato experiment and was begun at the same time, September 26, 1931. The tubers used were free from vascular discoloration and were surface sterilized. The soil temperatures used were 16° to 19°, 18° to 19° (see description of 2nd tomato experiment for an explanation regarding these two), 20°, 22°, 24°, and 26°. Two inoculated and 2 noninoculated cans were kept at each soil temperature. The inoculum was similar to that used in the 2nd tomato experiment, *i.e.,* a mixture of *Verticillium albo-atrum* and *V. dahliae.* The mean air temperature was 19°.

The growth of both inoculated and noninoculated potato plants was rather weak and spindly, and the leaflets were below the normal size. There was little difference visible between the growth of the noninoculated plants at any of the different soil temperatures used. Signs of disease appeared after very irregular intervals, as is shown in table 6. They were in many cases hard to distinguish from the gradual senescence, yellowing, and withering of the leaves from the lowest up, which took place in non-

TABLE 6.—*Influence of soil temperature on severity of Verticillium hadromycosis, length of incubation period, and relative pathogenicity of V. alboatrum and V. dahliae to the potato*

	Soil temperatures (mean air temperature = 19°)					
	16°	18°	20°	22°	24°	26°
Total weight in grams of tubers from noninoculated plants	99.0	98.0	90.0	97.0	84.5	80.5
Percentage of reduction in weight of tubers from inoculated plants	58	60	34	46	18	10
Estimated percentage of reduction in growth of tops of inoculated plants after 60 days' growth	60	85	70	30	30	20
Approximate incubation periods (in days) for each of the 4 inoculated plants at each soil temperature	33, 50, 55, 60	40, 40, 45, 45,	35, 45, 50, 55	33, 45, 55, 60	45, 50, 55, 60	55, 55, 60, 65
Average incubation period (days)	50	42	46	48	52	59
Results of isolations from inoculated plants	*V. albo-atrum* alone from 3 plants, *V. albo-atrum* + *V. dahliae* from 1 plant	*V. albo-atrum* alone from 2 plants, *V. dahliae* alone from 2 plants	*V. dahliae* alone from 1 plant	*V. albo-atrum* + *V. dahliae* from 1 plant, *V. dahliae* alone from 2 plants	*V. dahliae* alone from 4 plants	*V. dahliae* alone from 4 plants

inoculated potato plants grown in pots in the greenhouse under unfavorable conditions and also in the noninoculated plants of the tank experiment towards the end of their growth. Thus, the times given for the first appearance of symptoms are subject to an error of 5 days or so, due to this uncertainty. The greater length of incubation period in these plants than in the tomatoes or eggplants previously mentioned is accounted for, at least in part, by the fact that in the latter cases seedlings 2 or 3 in. high were planted and in the former case portions of tubers that took much longer to attain an equivalent development of aerial parts. The incubation periods for each of the 4 inoculated plants at each single soil temperature varied considerably (between 33 and 60 days in 2 cases). The disease appeared most rapidly at 18°, taking slightly longer at lower and higher soil temperatures. These results, however, are of doubtful significance.

After about 60 to 65 days the noninoculated plants ceased growth, probably owing to diminishing day length and light intensity; their leaves began to die off slowly from the lowest up, much as did those of the inoculated plants. Three noninoculated plants in which this process began a few days earlier than in the rest were plated, but all proved sterile.

Platings were made during the course of the experiment from the potato plants that developed symptoms of the disease. Though the disease was longer in appearing, it seemed to kill the plants off more rapidly than in the case of tomatoes or eggplants under similar conditions. All the leaves on a plant would be withered and the stem beginning to dry up from the tip downward within about 10 to 14 days of the first signs of disease. Plants were removed for plating while the upper leaves were still alive. Verticillium was isolated from the stem at least 3 in. above ground level, the stem and uppermost leaves being still alive and turgid, from one or more plants at each soil temperature used. The fungus was obtained in every case in which its isolation was attempted. The results of these reisolations are summarized in table 6. Microsclerotia were formed abundantly in stem-tissue cultures from the higher soil temperatures; dark hyphae alone developed in cultures from 3 out of 4 plants grown at 16° soil temperature and from 2 out of 4 plants grown at 18°. Each of 10 single-spore cultures from one 18° plant proved to be *V. albo-atrum*, and each of 10 from one 26° plant proved to be *V. dahliae*. While representing only a few isolations, these results would seem to indicate that *V. albo-atrum* is more actively parasitic on the potato at low soil temperatures and *V. dahliae* at high soil temperatures.

An estimate of the relative amounts of reduction in the aerial growth of inoculated potato plants, expressed as percentages of the amounts of aerial growth made by the noninoculated plants, was made at the end of 60 days' growth and is recorded in table 6. By December 9, after 74 days'

growth, the noninoculated plants were beginning to die; the tubers formed in each can were removed from the soil and weighed. It is thought that the weights thus obtained give the best available expression of the relative total amounts of growth per can, as the aerial parts of diseased plants were removed at various times during the last 2 or 3 weeks of the experiment for reisolation work. The results of the weighings are given in table 6. It is evident from the symptoms developed, the reisolation of Verticillium from the stems, and the reduction in weight of tubers, that there was some disease development at all the soil temperatures used, from 16° to 19° to 26°. The reductions in weight of the tubers agree with the other data in indicating slightly less severity of disease at soil temperatures of 24° and 26° than at 22° and below. Possibly the upper limit is higher than 26°, as no plants were grown above 26°, but the decrease in amount of disease at 26° gives reason to believe that little or no disease development would occur above this temperature. None of the tubers showed any sign of vascular discoloration: 2 tubers from each inoculated can were surface sterilized and pieces cut from them kept in a moist chamber, but no fungal growth appeared. Verticillium had readily been isolated by the writer on previous occasions by the same method from tubers of naturally diseased plants.

It is thought likely that under conditions more favorable to the healthy development of the potato plant and perhaps with a lower concentration of inoculum in the soil, the maximum soil temperature for the development of symptoms might be lower. This hypothesis is suggested by the writer's failure to produce disease symptoms in vigorous field-grown plants, although soil and air temperatures throughout a large part of the growing period were below those at which the disease was produced in the greenhouse. The same applies in the case of the tomato. In other words, it seems likely that the temperature relations of the disease are dependent to some extent upon the vigor of the host plants. Therefore, results gained from soil-temperature-tank investigations do not necessarily hold for field conditions in this case. Further evidence bearing upon this point will be discussed under the next heading.

Temperature Relations in the Field

Under the heading "Pathogenicity of *Verticillium dahliae* and *V. albo-atrum*—Field Experiments" it was pointed out that artificially inoculated eggplants, tomatoes, and potatoes, grown in the field during 1930 and 1931, failed in many cases to develop disease symptoms, though the temperatures prevailing during the greater part of the growing periods were found favorable for disease in plants grown in soil-temperature tanks. Somewhat similar observations have been recorded by Gratz (10). He describes

experiments repeated over several seasons, in which potato tubers infected with Verticillium were collected in Maine; half of these were grown in Maine and half in Florida. The Maine crops contained 25 to 30 per cent of severely diseased plants, while the Florida crops produced 0 to 3 per cent of diseased plants, none of which showed more than very slight symptoms. The temperatures during the first ⅔ of the Florida potato season do not differ significantly from those for the corresponding period in Maine and are well below the maximum for the fungus; Gratz considers that this evidence points to the likelihood of some factor or factors other than temperature being involved in the failure of the disease to appear in Florida. It should be noted, however, that during the last 3rd of the potato-growing season, temperatures are falling in Maine but rising in Florida.

In figure 8, the weekly mean air and soil temperatures during the growing

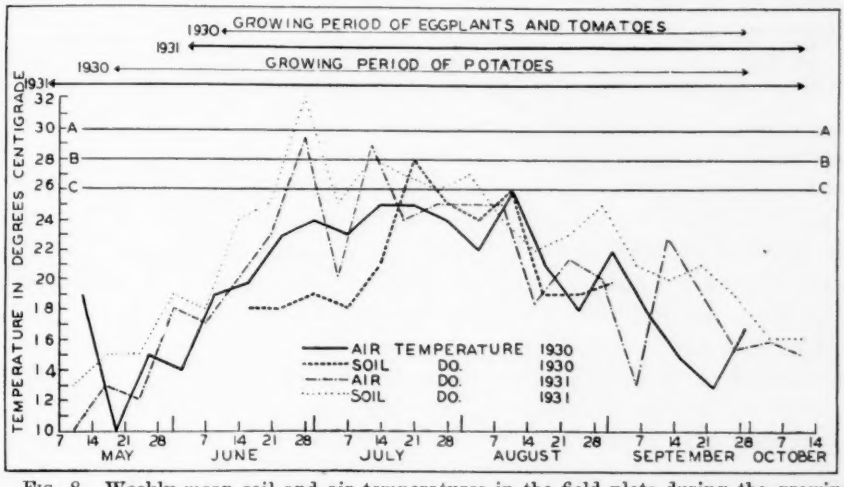

FIG. 8. Weekly mean soil and air temperatures in the field plots during the growing periods of 1930 and 1931, compared with the experimentally derived maxima for the occurrence of Verticillium hadromycosis. The maximum soil temperatures at which disease occurred in eggplants grown in soil-temperature tanks are indicated by the lines A (when air temperature was 22°) and B (when air temperature was 31°). The maximum soil temperature at which disease occurred in tomatoes and potatoes in tanks at 20° air temperature is indicated by the line C. The mean temperature for each week was plotted at a point corresponding to the end of that week.

periods of 1930 and 1931 are plotted for comparison with the highest soil temperatures at which distinct disease symptoms were obtained in the tank experiments. The mean temperatures in figure 8 were derived by averaging the 2-hourly readings of thermographs operated amongst the plants 12 in. above and 4 in. below ground level, with the following exceptions for

periods during which no thermograph was available. The air temperatures up to the middle of June, 1930, and the end of June, 1931, were derived from the United States official weather reports for Madison. The soil temperatures up to the end of June, 1931, were obtained by averaging thermometer readings taken daily at 8:00 A. M. and 7:00 P. M. The values thus obtained are only an approximation of the true weekly means, but it is thought they are more likely to be above than below the true mean temperatures for the following reasons. The bulb of the thermometer was placed 4 in. below soil level in unshaded ground (the roots of the plants would be partly shaded) and the morning reading was not taken till some time after sunrise, so that usually the ground would have warmed up appreciably from its night temperature before the reading was taken. Thus, if the true mean soil temperatures about the roots of the plants differed from the approximate values plotted in figure 8, they were probably lower and, therefore, there would be all the more reason to suppose that disease should have occurred.

In 1930 the weekly mean temperatures throughout the whole of the growing period were well below the maximum for the disease in eggplant, and nearly all the inoculated plants showed moderate symptoms of disease. In 1931 it was only during the last week in June and beginning of July that the weekly mean temperature exceeded the experimental maximum for the disease; during July the soil and air temperatures averaged 27° and 26°, respectively, but throughout the remainder of the growing period they were near or below the optimum for the fungus. Yet, about ⅓ of the 140 eggplants inoculated did not differ appreciably from the noninoculated plants, and most of the remainder showed symptoms that were very mild as compared with those obtained in the tank experiments. It would seem from the results of the tank experiments that more severe disease than this might have been expected to occur in the field. It is not thought that too low temperatures prevented its occurrence, as severe symptoms appeared at a soil temperature of 12° in the tank experiments.

No symptoms appeared in tomato plants except slight vascular discoloration at the base of the stem produced by 6 out of 9 cultures of Verticillium in 1930 and by 1 out of 7 cultures in 1931. The potatoes were unaffected in 1930; in 1931, 2 out of 7 cultures produced slight stunting and vascular discoloration, while the remainder had no effect. In no case were such severe disease symptoms produced in tomatoes or potatoes in the field as were obtained at a constant soil temperature of 26° and an almost constant air temperature of 20° in the tank experiments, using a mixture of *V. dahliae* and *V. albo-atrum* as inoculum. In 1930 the weekly mean soil temperatures for the first 5 weeks after transplanting the tomato seedlings into inoculated soil in the field were 18°, 18°, 19°, 18°, and 21°

(average 19°); the corresponding air temperatures were 20°, 23°, 24°, 23°, and 25° (average 23°). In the first tank experiment with tomatoes, symptoms first became evident within 18 days at soil temperatures of 20° and 24°, the mean air temperature being 23° in each case. The plants were severely diseased within 5 weeks. Similar results were obtained in the 2nd tank experiment at soil and air temperatures of 18° to 20°. In 1931 mean weekly temperatures between the 4th and 9th weeks after planting in the field approximated or exceeded the experimentally determined maximum for the disease in tomato, but there was ample time before and after the periods of heat to permit of the development of disease symptoms, according to the results of the tank experiments. The results of the single inconclusive tank experiment with potatoes also give reason to expect that more than the slight traces of disease produced in the field should have been obtained.

It therefore seems necessary to conclude that tank experiments do not give reliable data as to the maximal temperatures at which Verticillium hadromycosis may occur in the field and that conditions other than temperature are concerned in limiting its severity there. Of course, there was a wide variation between greenhouse and field conditions with regard to soil moisture, light intensity, and probably also humidity; exact data as to these factors are not available. Again, whilst the entire root system of each plant grown in the tanks was maintained at one constant temperature, the soil temperature in the field varied both diurnally and at different depths. Presumably the root systems of the field-grown plants were in a considerably healthier state than those of the tank-grown plants and better able to withstand injury to the finer rootlets, which may conceivably be a factor in injury due to Verticillium. No evidence on this point was obtained. Field-grown plants were naturally larger and more vigorous than those grown in the tank cans; it seems possible that the greater the general vigor of the host plant, the nearer to the optimum for the parasite must the temperature be before marked symptoms of hadromycosis can be produced. Bewley (4), speaking of tomatoes, writes, "There is a distinct relation between hardness of growth and susceptibility to wilt; the harder growing varieties and plants suffering from starvation or a severe check in the young stages being most susceptible to attack." Another possibility is that certain hosts may become liable to attack only when acted upon by predisposing factors at present unknown. Thus, Haenseler (11), speaking of Verticillium hadromycosis of the peach in New Jersey, says "It is very probable that winter injury is a necessary predisposing factor and that the organism is unable to infect a normal tree."

DISCUSSION

The apparent discrepancies between the reports of different workers on this disease are probably due in most cases to two main factors, the variability of the pathogen and the imperfectly understood influence of environment upon the host-parasite relations.

The variability of the pathogen has led to a still unsettled disagreement as to the best mode of taxonomic treatment of the vascular Verticillia. Most cultures described in the literature and 45 out of the 46 representative cultures examined by the writer fall into 2 classes, those that form microsclerotia and those that form dark hyphae without microsclerotia. There are some types that do not readily form any dark mycelium. Many of these will do so on appropriate treatment. A new species, *Verticillium dahliae*, was named by Klebahn to include the microsclerotial type, separating it from the type forming dark hyphae, only. Those who agree with Klebahn apply the designation *V. albo-atrum* to the latter type, alone, but it is often used in general literature to include both types. In addition to *V. dahliae*, several other species of vascular Verticillia have been named, *e.g.*, *V. ovatum* B. and J., *V. tracheiphilum* Curzi, etc., the claims of which to specific rank have been questioned by recent authors. The writer's study deals only with *V. dahliae* and *V. albo-atrum* (using the latter term to include types forming dark hyphae alone).

The classification of these 2 groups as separate species appears to the writer to be both useful and practicable. As pointed out earlier in this paper, the type of dark mycelium formed in culture provides a convenient means of distinction. The usefulness of the distinction depends on the extent to which the 2 groups differ in significant physiological characters, *i.e.*, the need for convenient distinction between them. Probably the most important distinctive physiological characters of these fungi are their pathogenicity and temperature relations. Their maximum temperatures are of particular importance in determining the climatic conditions under which disease may occur. The writer's studies agree with those of previous workers in showing that *Verticillium dahliae* is characterized by a distinctly higher maximum temperature than *V. albo-atrum* for growth in culture. The writer has shown this to apply also to disease production in the case of eggplant. Furthermore, *V. dahliae* is in most cases distinctly less pathogenic to certain hosts, *e.g.*, tomato, potato, and cucumber, than is *V. albo-atrum*, at least when the temperature is equally favorable for the growth of either species of fungus (though certain cultures of *V. dahliae* are more pathogenic to tomato than are certain cultures of *V. albo-atrum*). The work of Berkeley, Madden, and Willison (2) indicates that this difference in pathogenicity is more marked in the case of the cucumber.

The most important environal factor influencing the disease appears to be temperature. The general impression gained from the literature is that little disease is likely to occur above 25°. Soil-temperature-tank studies conducted by the writer showed that the maximum soil temperature for disease was about 30° in the case of eggplants inoculated with *Verticillium dahliae* and about 26° in the case of tomatoes and potatoes inoculated with a mixture of both species, the air temperature being about 20° in each instance. Thus there is a marked difference in maximum temperature for the disease in different hosts. The eggplant is more severely attacked than the tomato or potato at temperatures favorable for disease in any of these hosts. Possibly this accounts for the higher maximum temperature for disease in eggplants.

Field observations indicate that the disease may fail to develop in artificially inoculated field-grown plants when the temperature conditions are such as would permit of severe disease development in soil-temperature tanks. A given strain of *Verticillium dahliae* was found to be less pathogenic to vigorous, rank-growing tomato plants than to stunted plants. These facts and similar observations recorded in the literature are interpreted by the writer as suggesting that the host-parasite relationship involves a continual competition between the host and the parasite. Under this hypothesis any factor that retards the growth of the parasite more than that of the host, such as high temperature, will diminish the severity of disease. This diminishing of disease will be more marked when the host is in a vigorous condition than when the host is weak. It will also be more marked in hosts such as tomato and potato than in eggplant, the former two being less susceptible than the latter under optimum temperature conditions for the fungus. Similarly, any factor increasing the vigor of the host plants more than that of the fungus will lessen the amount of disease. Apparently, the maximum temperature for disease depends not merely on the maximum for growth of the fungus but also on the susceptibility of the host, which, in turn, depends on the species and general state of health of the plants concerned.

In the case of the potato, the somewhat fragmentary evidence available indicates that Verticillium is a comparatively weak parasite on this host. The occurrence of disease seems quite erratic and so dependent on environal conditions that the results of greenhouse experiments are of very limited utility in explaining the occurrence or absence of disease in the field. The environal relations of the disease in this host particularly are in need of further study. In the writer's opinion, the physiology of the fungus in the soil and of the potato plant under field conditions (including the possibility of factors predisposing the host to the disease) should receive careful attention in such a study.

SUMMARY

The taxonomy of the vascular Verticillia is briefly discussed. It is thought that the important differences in pathogenicity and temperature relations between the types differentiated by Klebahn as *Verticillium dahliae* and *V. albo-atrum* warrant their treatment as separate species.

Eight cultures of *V. albo-atrum* and 37 cultures of *V. dahliae* from a number of different hosts and localities were studied. As measured by rate of increase in colony diameter, the average growth rate of *V. albo-atrum* approximately equalled that of *V. dahliae* at 22° but was reduced to about ¼ that of *V. dahliae* at 28°. No growth of *V. albo-atrum* was obtained at 30°, while all cultures of *V. dahliae* tried at this temperature gave varying amounts of growth. The optimum for *V. albo-atrum*, according to the literature, is usually about 23°, but 1 culture of *V. dahliae* gave slightly better growth at 28° than at 22°.

Of 15 recorded hosts inoculated with *V. dahliae* under parallel field conditions, eggplant, okra, cotton, snapdragon, and rose appeared most susceptible; wild sumac, blackberry, and raspberry displayed mild symptoms; potato, tomato, pepper, cucumber, dahlia, lupin, and sweet pea showed no symptoms; though Verticillium was reisolated from potato, tomato, and dahlia.

Of 8 cultures of *V. albo-atrum* tested in the greenhouse for pathogenicity to potted tomatoes, 6 were severely pathogenic and 2 caused little or no disease. Nine out of 37 cultures of *V. dahliae* caused moderate to severe disease symptoms, and the remaining 28 displayed little or no pathogenicity to tomatoes under the conditions of the experiment.

In soil-temperature-tank experiments with eggplant, *V. dahliae* produced marked disease symptoms at soil temperatures of from 12° to 30°, but not at 32°; *V. albo-atrum* caused disease at 28° and below, but not at 30°. The air temperature was between 19° and 23° in each case. At 28° to 31° weekly mean air temperature, no disease was produced by *V. dahliae* above 28° soil temperature, and the symptoms at soil temperatures from 22° to 28° were much milder than in the previous experiments, disappearing towards the end of the experiment as the mean air temperature gradually increased.

Variation of soil moisture between 45 per cent and 95 per cent of the moisture-holding capacity had no appreciable effect on severity of disease in eggplant, except a slight lengthening of the incubation period and reduction in severity of disease with the highest 2 soil moistures at 28° soil temperature, probably due to the greater vigor of the host.

Severe disease in tomatoes was caused by *V. albo-atrum* up to 24° soil temperature, slightly less at 26°, only very slight symptoms at 28° and

none at 30° (air temperature = 20°). The culture of *V. dahliae* used caused very slight or no symptoms and no appreciable reduction in growth at soil temperatures from 16° to 30°, though it was reisolated from plants at 26° and 28° soil temperature. Higher air temperatures hindered the appearance of disease at favorable soil temperatures, as with the eggplant.

In a single tank experiment with potatoes, disease occurred from 16° to 26° soil temperature (air temperature 19°) with a mixture of both species of fungus as inoculum.

The difficulty experienced in obtaining the disease in the field at temperatures below the experimentally derived maxima leads to the belief that some factor or factors other than temperature must have lessened the susceptibility of the field-grown plants to the disease. It is suggested that vigorous plants may be resistant to the disease at temperatures favorable for disease in weaker plants.

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THE PREPARATION AND PROPERTIES OF PENTATHIONIC ACID AND ITS SALTS; ITS TOXICITY TO FUNGI, BACTERIA, AND INSECTS

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For the last century, various types of sulphur sprays and dusts have been used as crop protectants with varying degrees of success. The common lime-sulphur sprays and the 300-mesh sulphur dusts are now in universal use as fungicides. Sulphur dusts, in which the particles are very fine and readily oxidized, have proved satisfactory in the control of many plant diseases. But these dusts have apparently insufficient fungicidal properties to control fungous diseases such as Brooks spot and the bitter rot of apple.

Sulphur sprays and dusts have not been used extensively to combat bacterial diseases of plants. This is probably due, at least in part, to the difficulty of applying sprays and dusts to infected parts and at a time when they will not be injurious to the host. Furthermore, it may be that sulphur, in the forms in which it is available, is not sufficiently toxic to satisfactorily control such bacterial diseases as fire blight of apple and bacterial spot of peach.

The use of sulphur as an insecticide has largely been confined to the lime-sulphur sprays. The theory that such sprays are effective because of their causticity has recently been questioned by O'Kane and Conklin (7). Although lime sulphur sprays have given good control of some scale insects, there appears to be, at present, no sulphur insecticide that will satisfactorily control aphid infestations.

The results of laboratory tests made by Young (15) show that pentathionic acid, an oxidation product of sulphur, is toxic to fungi. The more recent tests made by Liming (6) support Young's theory. Lack of control with sulphur of some fungous and bacterial diseases and insect infestations may be due to an inadequate amount of pentathionic acid. It therefore appears that a spray or dust with, or capable of producing, a large amount of the acid would be a good crop protectant against most diseases and insect attacks.

The experimental work presented herein is divided into three main subjects. The first part deals with method of preparation, composition, and properties of three pentathionate salts and pentathionic acid. In the second part are given methods of preparation, composition, and the properties of

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a colloidal sulphur paste, together with some suggestions for its commercial preparation. The third part includes the results of tests to show the effect of pentathionic acid on 9 pathogenic fungi, 3 bacterial plant pathogens, and 3 common species of plant aphids. The results of some preliminary tests of the toxicity of colloidal sulphur also are given.

HISTORICAL REVIEW

Pentathionic acid was discovered by Wackenroder to be the principal acid formed by the reaction of sulphur dioxide and hydrogen sulphide in water. The difficulty encountered in preparing the pure salts led Spring (12) to question the interpretation Wackenroder made of his own results. Spring regarded pentathionic acid as a solution of sulphur in tetrathionic acid. Later, Debus (2) prepared several pentathionate salts and definitely proved the existence of the acid. He gave the structural formula, $H \cdot S_2 \cdot SO_2 \cdot OS \cdot SO_2 \cdot O \cdot H$, to indicate the arrangement of the sulphur atoms in the molecule.

The acid occurs in hydrophilic sulphur sols, plastic sulphur, and ground sulphur. The nature of 2 types of hydrophilic sulphur sols has been investigated by Freundlich (3, pp. 615–625). He found that 1 gm. of sulphur from the Wackenroder solution carried about 0.145 millimol of pentathionic acid; whereas 1 gm. of sulphur, formed when sodium thiosulphate is decomposed by an acid, was found to carry about 0.470 millimol of the acid. Babson (11) found that when thiosulphuric acid is mixed with black soil about 80 per cent of it is changed to pentathionic acid. Jablczynski and Frenkenberg (4) reported that the rapidity of decomposition of thiosulphuric acid is increased by the presence of colloidal sulphur.

Another type, a hydrophobic sulphur sol, prepared by adding an excess of water to an alcoholic solution of sulphur, contains, according to Freundlich (3), no pentathionic acid.

Plastic sulphur has pentathionic acid associated with it. Freundlich (3) believed that the pentathionic acid that is mixed with the plastic or amorphous sulphur greatly inhibits its transformation to crystalline sulphur. He pointed out that the conditions under which amorphous sulphur is prepared also favor the formation of pentathionic acid.

The occurrence of pentathionic acid on ground sulphur was reported by Young and Williams (16). They suggested that the natural oxidation of sulphur gives a continuous yield of the acid but that the amount is too small to bring about hydration of the sulphur particles. The later work of Roach (11) showed that when sulphur is added to black soils about 6 per cent of it is oxidized to pentathionic acid.

Three general methods have been employed in the preparation of pentathionic acid. The sodium thiosulphate-hydrochloric acid method is

probably the most satisfactory one of the three. By this method it is possible to prepare a solution containing principally pentathionic acid and its sodium salt. Under favorable conditions and in the presence of a small quantity of arsenious acid, the complete conversion of thiosulphate into pentathionate is theoretically possible. Bassett and Durrant (1) give the following equations to represent these reactions.

$$H_2S_2O_3 + H_3AsO_3 \longrightarrow H_2SO_3 + H_3AsO_3S$$
$$H_2S_3O_6 + H_3AsO_3S \longrightarrow H_2S_4O_6 + H_3AsO_3$$
$$H_2S_4O_6 + H_3AsO_3S \longrightarrow H_2S_5O_6 + H_3AsO_3$$

The sulphur dioxide-hydrogen sulphide method consists of passing hydrogen sulphide into a concentrated solution of sulphurous acid. According to Debus (2), the resulting solution is a mixture of the polythionic acids. Raschig (8) and Riesenfeld and Feld (10) have given the following equations to explain the reaction.

$2SO_2 + H_2S \longrightarrow H_2S_3O_4$ Hypothetical acid
$H_2S_3O_4 + SO_2 \longrightarrow H_2S_4O_6$ Oxidation by sulphur dioxide
$H_2S_3O_4 + 6SO_2 \longrightarrow H_2S_8O_6$ Oxidation by sulphur dioxide
$5H_2S_3O_4 \longrightarrow 3H_2S_5O_6 + 2H_2O$ Polymerization

The amount of acid formed per unit volume by this method is so small that considerable difficulty is encountered in reducing the solution to the concentration necessary for crystallization of a salt in presence of a base.

When sulphur monochloride in aqueous solution is added in small quantities to a pasty mixture of barium thiosulphate and water, a solution is obtained that exhibits all of the properties of pentathionic acid. Spring (12) believed that the product of these reactions is a mixture of polythionic acids.

Young (15) tested the effect of hydrophilic sulphur on 8 pathogenic fungi and found it toxic only between pH 4.2 and 5.4. In his experiments, the colloidal sulphur was coagulated and centrifuged from the "mother liquid" and then peptized in distilled water. Tisdale (13) tested the toxicity of sulphur precipitated from lime sulphur solution, along with the hydrophilic sulphur sols and found them toxic to 8 pathogenic fungi. He reported that aeration and slight desiccation do not destroy the toxic factor.

The results of toxicity tests made by Liming (6) show that, of the acids of sulphur, the polythionic acids are the most toxic to spores of *Sclerotinia cinerea*. Pentathionic acid prepared from the barium salt was found to be toxic in varying degrees to several pathogenic fungi. Filtrates from ground sulphur that contained pentathionic acid were likewise found to be toxic to spores of *S. cinerea*.

THE PREPARATION AND PROPERTIES OF PENTATHIONATE SALTS AND OF PENTATHIONIC ACID

For detailed and exact studies of the effect of pentathionic acid on biological materials, acid solutions from pentathionate salts were used. Such solutions, the exact composition of which is known, are, for laboratory test, highly preferable to commercial materials. Owing to the constant composition of the recrystallized salts, it is possible to prepare at all times solutions that are alike with reference to the composition of the acid in them. The results of toxicity tests in which such acid solutions are used are more reliable, and the results of various tests can be satisfactorily compared with each other.

PERPARATION OF SODIUM, BARIUM, AND POTASSIUM PENTATIONATES

Raschig's method, which employs the reaction between a thiosulphate and an acid in the presence of arsenic, was used in this investigation. The procedure was modified in some respects in an effort to increase the yield of pentathionic acid. One hundred and twenty-five grams of sodium thiosulphate were dissolved in 150 cc. of water, and then to this 2.5 gm. of arsenous oxide, dissolved in a small amount of sodium hydroxide, were added. The temperature of the solution was reduced to $-10°$ C. in a salt-ice bath. Then, 200 cc. of concentrated hydrochloric acid, likewise cooled to $-10°$ C., were added, 5 cc. at a time. The solution was vigorously stirred and kept at the low temperature during the procedure. The resultant precipitate of sulphur, arsenous sulphide, sodium chloride, and sodium sulphate was removed by filtartion.

The filtrate was placed in a porcelain evaporating dish and set before an electric fan. After about 4 hours fanning, the solution was again filtered to remove the precipitate. This procedure was repeated three times, at the conclusion of which the solution had been reduced in volume from 350 cc. to 100 cc., and all the free sulphur and sodium chloride had been removed. It was necessary, during this procedure, to keep the temperature of the solution below 35° C. to inhibit the separation of sulphur.

About 100 cc. of absolute alcohol were added to crystallize the sodium pentathionate. It was observed that if the alcohol was added with sufficient care a layer formed on top of the solution and crystallization took place readily. The next day the sodium pentathionate was removed, thoroughly washed with absolute alcohol, and dried at room temperature.

The filtrate was freed of alcohol by evaporation and then divided into two equal parts. To one part was added 50 cc. of a saturated solution of barium acetate containing 3 per cent acetic acid. A precipitate of barium sulphate was formed, thus removing from solution all of the sulphuric

acid formed by the thiosulphate-acid reaction. The precipitate was removed and saved for quantitative determination. The solution was concentrated by fanning until its volume was again about 50 cc.; to this was added an equal volume of absolute alcohol. The same method of crystallization, filtration, and washing was followed here as described above for the sodium salt.

The other part was treated with just enough barium hydroxide to remove the sulphuric acid. Then, 50 cc. of a 20 per cent solution of potassium acetate plus 3 per cent acetic acid were added. From this point on the procedure was the same as that followed in the preparation of the barium pentathionate.

The filtrates from the two parts were combined and the amount of pentathionic acid that was left in solution was determined. The amounts of free sulphur and various compounds from two such mixtures are given in table 1.

TABLE 1.—*Quantities of free sulphur and sulphur compounds produced by the reaction of 250 gm. of sodium thiosulphate with hydrochloric acid. (Combination of products from two 125 gm. mixtures as recorded in text)*

Sulphur compounds	Compounds	Sulphur	Total S. in the compounds
	gm.	*gm.*	*Per ct.*
As_2S_3	3.07	1.20	1.9
$BaSO_4$	6.13	.84	1.3
Na_2SO_4	trace	.....	.....
$Na_2S_5O_6 \cdot 4H_2O$	31.90	13.81	21.2
$BaS_5O_6 \cdot 3\frac{1}{2}H_2O$	19.78	7.00	10.8
$K_2S_5O_6 \cdot 1\frac{1}{2}H_2O$	21.31	9.45	14.6
$H_2S_5O_6$	12.12	7.52	11.6
Total	.....	39.82	61.4
Free sulphur (theoretical)	24.76	24.76	38.6
$Na_2S_2O_3 \cdot 5H_2O$	250.00	64.58	.....

The results show that about 60 per cent of the sulphur in the thiosulphate molecule formed pentathionic acid. The difference in the amounts of the various pentathionate salts is not significant, as the amount of the base added and the time were probably determining factors.

The Physical Properties of Pentathionate Salts

All three of the pentathionate salts are stable when kept dry at room temperature. Samples of the salts were kept in moisture-proof containers

for nearly a year, and no important decomposition occurred. A sample of sodium pentathionate, kept on a watch crystal in a desk in the laboratory for 6 months, showed no signs of decomposition.

Samples kept in a moist atmosphere, however, began to break down almost immediately, gave off sulphur dioxide, and became yellow because of formation of free sulphur. Likewise, samples that were not washed thoroughly with absolute alcohol and completely dried were unstable.

Samples of the 3 pentathionate salts were examined by V. H. Morris, Biochemist, Department of Agronomy. The descriptions and drawings of the crystals, and their optical properties, as contributed by him, are given in the following paragraphs.

The optical and crystallographic properties of the potassium and barium salts of pentathionic acid have already been described by C. W. Mason (14, pp. 398–400). The samples of the same salts, prepared as described above, were found to have identical properties.

Sodium pentathionate. The crystals obtained were lamellar in habit, very thin plates that tended to adhere in groups and clusters. They belong to the orthorhombic system. The plates usually are considerably elongated in the direction designated as *c* and flattened parallel to *c* and *b* (Fig. 1, A).

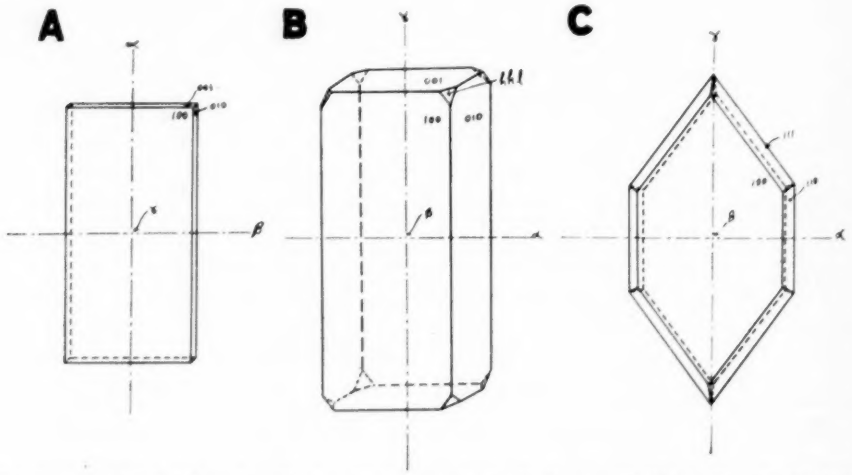

FIG. 1. Crystal habit and optical orientation of: A. Sodium pentathionate. B. Barium pentathionate. C. Potassium pentathionate.

The plates exhibit parallel extinction in all directions. The axial plane is 010; the obtuse bisectrix is *a* and the optic normal *b*. The sign of elongation is negative, as is also the optical character. The refraction indices by the immersion method are: $\alpha = 1.551$; $\beta = 1.605$; and $\gamma = 1.610$.

The above plates, dissolved in water and recrystallized by the addition of water, were obtained as imperfect, tapering, rectangular tablets. The thicker crystals do not exhibit complete extinction, and normal interference figures could not be obtained. The refraction indices of α and β are the same as those of the plates described above and are in the same orientation. These crystals appear to be aggregates of plates similar to those above but adhering together with a definite orientation.

Barium pentathionate. The crystals are most commonly observed as rectangular tablets belonging to the orthorhombic system. They usually are elongated in the direction taken as *c* and the corners are often truncated by small bipyramids (Fig. 1, B).

The tablets exhibit parallel extinction in all directions, and biaxial interference figures may easily be obtained. The axial plane is 100; the optic normal is *a*, and *c* is the acute bisectric. The sign of elongation is positive, as is also the optical character of the crystals. The refraction indices by the immersion method are $\alpha = 1.620$; $\beta = 1.640$; and $\gamma = 1.670$.

Potassium pentathionate. The crystals belong to the orthorhombic system, and the most common crystal habit is illustrated in figure 1, C. If the crystallographic axes are located so that the flattening is parallel to *b* and *c*, the largest faces may be designated 100, and these are beveled by 110 and 111. The crystals are thus hexagonal, with terminal angles of about 74°.

With the crystals oriented as illustrated, the axial plane is 100; the acute bisectric is *c* and the optic normal is *a*. The sign of elongation and the optical character of the crystals are positive. The refraction indices by the immersion method are: $\alpha = 1.570$; $\beta = 1.630$; and $\gamma = 1.658$.

Quantitative Analysis of Pentathionate Salts

The amounts of sulphur and bases in sodium, barium, and potassium pentathionates were determined. In all cases the sulphur was oxidized to a sulphate and measured gravimetrically as barium sulphate. In the case of the barium pentathionate, the barium was precipitated from an aqueous solution as a sulphate and weighed. The amount of base in each of the other salts was determined by oxidizing the sulphur to a sulphate, evaporating the solution to a constant weight, and measuring the residue as sodium or potassium sulphate, as the case might be. Only a brief outline of the procedure for each of the salts is given here.

Sodium pentathionate. A small amount of the salt (0.3826 gm.) was dissolved in 10 cc. of distilled water. Five cc. of a 10 per cent bromine solution were carefully added. The solution was allowed to stand 15 minutes, and then 10 cc. of nitric acid were added. It was warmed until the reaction ceased and the bromine was volatilized. The volume was made up

to 30 cc. and then divided into 2 equal parts. One part was evaporated to dryness, treated with 10 cc. of 10 per cent hydrochloric acid, and dried again to drive off the nitric acid. After the solution was diluted to about 200 cc., 5 cc. of 10 per cent hydrochloric acid, and then 50 cc. of 0.5 N. barium chloride were added. The resultant precipitate, barium sulphate, was collected on a weighed Gooch crucible, ignited, cooled in a desiccator, and weighed as barium sulphate.

The precipitate weighed 0.5927 gm. and therefore contained 0.0813 gm. of sulphur. Theoretically, such a sample should contain 0.0828 gm. The discrepancy of 0.0015 gm. was probably caused by an impurity such as sodium chloride.

The second portion was treated in the same manner as the first, except that sulphuric instead of hydrochloric acid was added. Then the solution was evaporated to dryness and constant weight in a weighted platinum dish. From the amount of the sodium sulphate (0.0774 gm.) thus formed, the amount of sodium was found to be 0.0241 gm. This amount is 0.0004 gm. more than the amount theoretically present in 0.1913 gm. of sodium pentathionate.

Barium pentathionate. By the method described above, the amount of sulphur in 0.3060 gm. of barium pentathionate was determined. From this sample 0.7842 gm. of barium sulphate was formed. The results indicate that the sample contained 0.1075 gm. of sulphur, an amount that is 0.0007 gm. less than the theoretical amount.

For the determination of the barium, 0.3060 gm. of the salt was dissolved in 10 cc. of water. To this solution 10 cc. of 5 per cent sulphuric

TABLE 2.—*Results of quantitative analyses of sodium, barium, and potassium pentathionates*

Pentathionate salts and molecular weights	Experimental results and calculations	Theoretical calculations
$Na_2S_5O_6 \cdot 4H_2O$ 370.30	× : .5927: : 32: 233.43	× : .1913: : 160.3: 370.3
	× equals 0.0813 gm. S.	× equals 0.0828 gm. S.
	× : .0744: : 46: 142.06	× : .1913: : 46: 370.3
	× equals 0.0241 gm. Na.	× equals 0.0237 gm. Na.
$K_2S_5O_6 \cdot 1\frac{1}{2}H_2O$ 361.53	× : .9264: : 32: 233.43	× : .2909: : 160.3: 361.53
	× equals 0.1270 gm. S.	× equals 0.1290 gm. S.
	× : .1413: : 78.2: 174.26	× : .2909: : 78.2: 361.53
	× equals 0.0634 gm. K.	× equals 0.0629 gm. K.
$BaS_5O_6 \cdot 3\frac{1}{2}H_2O$ 453.17	× : .7842: : 32: 233.43	× : .3060: : 160.3: 453.17
	× equals 0.1075 gm. S.	× equals 0.1082 gm. S.
	× : .1582: : 137.37: 233.43	× : .3060: : 137.37: 453.17
	× equals 0.0931 gm. Ba.	× equals 0.0928 gm. Ba.

acid were added. From the weight of the precipitated barium sulphate (0.1582 gm.), it was calculated that 0.0931 gm. of barium was present. The difference between this and the theoretical value (0.09 gm.) is not significant.

Potassium pentathionate. A 0.5818-gm. sample of the salt was used in the determination of the amounts of sulphur and potassium by the same methods employed in the analysis of the sodium salt. Only 0.9262 gm. of barium sulphate, carrying 0.1270 gm. of sulphur, was obtained from 0.2909 gm. of potassium pentathionate.

From the other half of the sample, 0.1413 gm. of potassium sulphate was obtained. This amount of potassium sulphate contains 0.0634 gm. of potassium, which is 0.0005 gm. more than the amount actually present in 0.2909 gm. of the pure salt.

The results of the 6 quantitative analyses and the formulae used in the calculations are given in table 2.

The Preparation and Physical Properties of Pentathionic Acid

A brief outline of 3 general methods for the preparation of pentathionic acid has been given in the historical review of this paper. One of these methods, the thiosulphate-acid reaction, has been described in detail in the section dealing with the preparation of the 3 pentathionate salts.

A concentrated solution of pentathionic acid, comparable in strength to that from which the pentathionate salts were prepared, slowly decomposed on long standing in the laboratory. Free sulphur and sulphur dioxide were the principal decomposition products. After standing 15 days, sufficient sulphur was formed and held in suspension to give the solution a yellow color. Pentathionic acid, which was prepared in dilute solutions from the barium salt, was quite stable. Only a trace of free sulphur and sulphur dioxide was found in the solution after standing 6 months.

The Analyses of Polythionic Acids

For quantitative analyses, a stock solution containing only the polythionic acids was prepared. For this solution, 1.5 gm. of barium pentathionate were dissolved in 16.4 cc. of water; to this were added 33.6 cc. of .2 N. sulphuric acid. The resultant precipitate of barium sulphate was removed by filtration. Qualitative tests showed that the solution contained principally pentathionic, some tetra- and trithionic, but no sulphuric, acids. The amount of each was determined in the presence of each other by 3 volumetric titrations.

The total amount of polythionates was found by measuring the acid formed from the double decomposition of mercuric chloride. A 10-cc. por-

tion of the stock solution was pipetted into 10 cc. of a 10 per cent solution of mercuric chloride. This was well mixed, allowed to stand 10 minutes, diluted to 50 cc., and heated to boiling. After cooling, 25 cc. of 10 per cent ammonium chloride were added to prevent the separation of sulphur in the subsequent titration. The solution was then titrated with .1 N. sodium hydroxide, methyl orange being used as an indicator. For this sample, 26.9 cc. of alkali were required to neutralize the acid formed by the mercuric chloride reaction.

The polythionates react with mercuric chloride, according to Kurtenacker and Bittner (5), as follows:

$$2\,S_3O_6 + 3\,HgCl_2 \rightarrow Hg_3S_2Cl_2 + 4\,Cl + 8\,H$$
$$2\,S_4O_6 + 3\,HgCl_2 \rightarrow Hg_3S_2Cl_2 + 4\,Cl + 8\,H + 2\,S$$
$$2\,S_5O_6 + 3\,HgCl_2 \rightarrow Hg_3S_2Cl_2 + 4\,Cl + 8\,H + 4\,S$$

The equations show that for every 6 mol. of polythionates there were 24 mol. of acid released. Then it follows that the total mols of the polythionates, $S_3O_6 + S_4O_6 + S_5O_6 = a/4$, in which "a" represents the mols of sodium hydroxide used in the titration. The results show that 0.3 gm. (the amount contained in 10 cc. of the stock solution) of the barium salt contains 0.6725 millimol of polythionates.

The total amount of tetra- and pentathionates was determined by the action of a sulphite on the acids. Another 10-cc. portion of the stock solution was treated with 10 cc. of a 10 per cent solution of sodium sulphite and then allowed to stand 10 minutes. To this were added 5 cc. of formaldehyde and 10 cc. of 10 per cent acetic acid. The solution was then titrated with .1 N. iodine, starch being used as an indicator. This solution required 13.1 cc. of .1 N. iodine to neutralize the thiosulphate formed by the reaction.

According to Raschig (8), the thiosulphate was formed by the following reactions:

$$S_4O_6 + SO_3 \rightarrow S_3O_6 + S_2O_3$$
$$S_5O_6 + 2\,SO_3 \rightarrow S_3O_6 + 2\,S_2O_3$$

The trithionate does not react with the sulphite. The equations show that one mol of iodine was required for each equivalent of tetrathionate, whereas two were required for each pentathionate. These conditions can be represented by $S_4O_6 + 2\,S_5O_6 = b$, where "b" is equivalent to the amount of normal iodine used. The results show that, in this case, "b" represents 1.31 millimol.

By another reaction the total amount of the tetra- and pentathionates was determined. But, unlike the sulphite reaction, each of the 2 higher thionates yields the same amount of thiosulphate. Ten cubic centimeters of the stock solution were diluted to 100 cc., and then 5 cc. of a 10 per cent

solution of potassium cyanide were added. After five minutes' standing, the solution was treated with 5 cc. of formaldehyde and 5 cc. of 10 per cent sulphuric acid. The solution was titrated immediately with .1 N. iodine, 6.65 cc. being required to neutralize the thiosulphate.

Kurtenacker and Bittner (5) state that the following reactions take place:

$$S_3O_6 + 3\,CN \rightarrow SO_3 + CNS$$
$$S_4O_6 + 3\,CN \rightarrow S_2O_3 + CNS$$
$$S_5O_6 + 4\,CN \rightarrow S_2O_3 + 2\,CNS$$

The first equation shows that the trithionate produced a sulphite. Due, however, to the presence of formaldehyde, this sulphite did not enter into the iodine reaction. By use of the equation, $S_4O_6 + S_5O_6 = c$, where "c" is the amount of normal iodine consumed, it is shown that there was 0.665 millimol of tetra- and pentathionates.

From the results of the 3 titrations, the amount of each of the 3 polythionates was calculated as follows:

$$a = S_3O_6 + S_4O_6 + S_5O_6 = 0.6725 \text{ millimol}$$
$$b = S_4O_6 + 2\,S_5O_6 = 1.3100 \text{ millimol}$$
$$c = S_4O_6 + S_5O_6 = 0.6650 \text{ millimol}$$

Therefore,

$$S_3O_6 = 0.6725 - 0.6650 = 0.0075 \text{ millimol}$$
$$S_4O_6 = 2(0.665) - 1.310 = 0.0200 \text{ millimol}$$
$$S_5O_6 = 1.310 - 0.665 = 0.6450 \text{ millimol.}$$

From the amount of pentathionic acid found in a 0.3 gm. sample, it was calculated that the salt contained 0.2923 gm. (0.645 times 453.17) of barium pentathionate ($BaS_5O_6 \cdot 3\frac{1}{2}H_2O$). Therefore, the salt was about 97.4 per cent barium pentathionate. Further, the total amount of sulphur accounted for in the 0.6725 millimol of the polythionic acids was 0.1065 gm. By gravimetric methods, 0.306 gm. of the salt was found to contain 0.1075 gm. of sulphur. From this, it was calculated that 0.3 gm. contained 0.1054 gm. of sulphur. The discrepancy of 0.0011 gm. is attributed to experimental error.

HYDROPHILIC COLLOIDAL SULPHUR

Hydrophilic colloidal sulphur has never been used extensively as a fungicide or an insecticide. Laboratory tests have shown that the spray is effective, but the cost of sulphur in this form always has been a limiting factor in its use. Usually, the colloidal sulphur has been coagulated, removed from the "mother liquid," and then dried. Tisdale (13) found that aged colloidal sulphur could not be easily suspended in water, and furthermore, he found that most of its toxicity to fungi was lost.

A sulphur paste was prepared in this laboratory by a method in which the coagulating and centrifuging were omitted and the colloidal properties and the toxicity of the sulphur were not materially reduced. A hydrophilic colloidal suspension, containing sulphur and pentathionic acid, was reduced by evaporation to a thick paste. The pentathionic acid, which stabilizes the colloidal suspension and which is toxic to fungi, was retained in the paste.

PREPARATION OF COLLOIDAL SULPHUR

The Wackenroder method, which employs the reaction of sulphur dioxide and hydrogen sulphide in water, was modified to some extent and used in the preparation of hydrophilic colloidal sulphur.

The amounts, 40 l. of sulphur dioxide, 20 l. of hydrogen sulphide, and 1 l. of water, were chosen arbitrarily for the experiment. One half of the sulphur dioxide was introduced into the water. The temperature was lowered to 10° C. and held near this point during the entire procedure. Then, 5 l. of gas, consisting of equal parts, by volume, of sulphur dioxide and hydrogen sulphide, were bubbled into the sulphurous acid at the rate of 10 l. per hour. The gas was then shut off, and the suspension was allowed to stand 2 hours. This procedure was repeated 8 times.

All of the gas that escaped from the reaction chamber during the process was collected over glycerine and later returned to the suspension. After all of the gas was consumed, the suspension was allowed to stand overnight. During this time some sulphur dioxide was formed. A large quantity of sulphur settled out, but the suspension remained sulphur yellow. More sulphur dioxide was formed when the suspension was removed from the ice bath. This gas was driven off by aeration and collected in an iodine solution for quantitative determination.

The suspension was transferred to a weighed porcelain evaporating dish and placed in front of an electric fan. During the evaporation process the temperature never exceeded 35° C. By this method the water was driven off until a thick paste, weighing 125 gm. was formed. At this time a faint odor of sulphur dioxide was noticeable. However, the amount was so small that it was not considered in the subsequent quantitative analyses.

Qualitative tests showed that the paste contained sulphur, and sulphurous (and possibly thiosulphuric), sulphuric, pentathionic, and traces of other polythionic acids. The amounts of the sulphur and the various acids were determined by the method described earlier in this paper.

The results of the quantitative analyses are tabulated in table 3.

The results from 3 experiments show that such a paste, on an average, is about 68 per cent sulphur, about 69 per cent of the sulphur being in a free state and 31 per cent, combined in ions of the various acids.

TABLE 3.—*Amounts of sulphur and sulphurous (and possibly thiosulphuric) sulphuric, and polythionic acids in 125 gm. of colloidal sulphur paste prepared from about 40 l. of sulphur dioxide, 20 l. of hydrogen sulphide, and 1 l. of water*

Test	Grams of sulphur in source materials			Grams of sulphur in the various constituents of the paste			
	SO_2	H_2S	Total S.	H_2SO_3	H_2SO_4	$H_2S_3O_6$	Free S.
1	57.1	28.5	85.6	0.05	0.13	26.06	59.21
2	56.7	28.5	85.2	0.03	0.08	26.02	58.74
3	58.2	28.5	86.7	0.07	0.17	26.49	59.19
Total	172.0	85.5	257.5	0.15	0.38	78.57	177.14
Percentage of sulphur				0.06	0.15	30.5	68.8

Stated in a more general way, the paste is composed of free sulphur (47 per cent), pentathionic acid with traces of other acids (34 per cent), and water (19 per cent).

PHYSICAL PROPERTIES OF COLLOIDAL SULPHUR

The sulphur dioxide, formed when the suspension was reduced to a paste, was apparently a decomposition product of the polythionic acids. The amount of the gas was greatly increased when the paste was left exposed to the air for a day. But, the formation of the gas almost ceased when the paste was sealed in an air-tight container. Paste that was exposed to air gradually lost its colloidal properties and was not easily peptized in water. Moreover, the sulphur when completely dried could not be freely suspended in water, even after much tedious grinding. When left in the dry state, the sulphur eventually crystallized into the rhombic sulphur form.

A suspension made up of 1 gm. of paste in 500 cc. of water was creamy white in appearance, free from the odor of sulphur dioxide, and very stable in nature. A suspension kept in the laboratory for 3 months retained its normal appearance and very little sulphur settled out. The colloidal properties were not destroyed by small amounts of lead arsenate and potassium fish-oil soap. It was necessary to dissolve the soap in the water before the sulphur paste was added.

COLLOIDAL SULPHUR PASTE FOR COMMERCIAL USE

The quantities of the materials used and the procedures followed throughout this investigation were chosen without consideration of the time involved. Preliminary tests had shown that, by the methods used, a maximum amount of pentathionic acid could be produced.

In some other tests a slight modification was made by bubbling hydrogen sulphide into a concentrated solution of sulphurous acid. It seemed prac-

ticable to then introduce more of each of the gases, the sulphur dioxide always being in excess, until a thick creamy suspension was formed. A great reduction in the time required for the evaporation was thus effected. It was found, however, that by this short method the relative amount of pentathionic acid was reduced. When, to hasten evaporation, the temperature was raised, the separation of sulphur was increased, but the time required for concentrating the suspension to a paste was greatly reduced. Whether or not the time and labor saved by the use of these methods compensate for the decrease in the relative amount of pentathionic acid in the paste has not been determined.

TOXICITY OF PENTATHIONIC ACID AND COLLOIDAL SULPHUR

Many tests were made to determine the effect of pentathionic acid on 3 biological forms—fungi, bacteria, and insects—all of which may be detrimental to agricultural crops.

Plant Pathogenic Fungi

Nine pathogenic fungi, each of which sporulated readily on potato-dextrose agar, were chosen for toxicity tests. In each case the fungus was transferred to the middle of an agar plate. The plate was then placed in a constant-temperature chamber until the fungus produced an abundant supply of conidia. About 10 cc. of a slightly buffered (pH 5.4) sodium hydroxide-phosphoric acid-mannite solution were poured onto the fungous colony and spread around with a glass rod. The suspension was then diluted with more of the solution until a low-power microscope field contained about 100 spores.

A solution of pentathionic acid, prepared from barium pentathionate, was made twice as strong as was desired for the toxicity tests. Two cubic centimeters of the spore suspension and the same volume of the acid were transferred to a test-tube. By means of a small glass rod a small drop of the spore suspension-acid was spread in a thin film on each of 4 cover slips. The slips were then inverted over glass rings contained in a Petri plate. The excess of the spore suspension-acid was poured into the bottom of the plate. By this means a vapor-pressure equilibrium was maintained within the germination chamber. The set was then placed in a constant-temperature chamber adjusted to 23° C.

With but two exceptions, the percentages of spore germination were determined at the end of 18 hours by counting individual spores under the low power of the microscope. *Verticillium albo-atrum* and *Phomopsis* sp. required 48 hours for satisfactory germination. A spore without, or with a very short dead, germ tube was recorded as not germinated.

The results of the tests show that pentathionic acid is very toxic to the spores of the 9 pathogenic fungi tested, whereas sulphuric acid of the same

TABLE 4.—*Toxicity of pentathionic acid to spores of pathogenic fungi in a buffer-mannite solution*

Pathogenic fungi that were tested	Germination		
	$H_2S_5O_6$		H_2SO_4 .0068 N.
	.0068 N.	.00068 N.	
	Per ct.	*Per ct.*	*Per ct.*
Sclerotinia cinerea	0.0	60.0	97.1
Phomopsis sp.	0.0	67.5	87.4
Verticillium albo-atrum	0.0	20.8	98.6
Graphium ulmi	0.0	8.7	70.9
Glomerella cingulata	0.0	71.3	91.3
Botrytis cinerea	0.0	97.0	98.3
Fusarium monifiliforme	3.3	43.9	89.1
Thielavia basicola	0.0	29.1	69.9
Phoma pomi	0.0	55.1	96.8

normality shows no marked toxicity to the spores of the organisms. The presence of mannite in the solution evidently made conditions more favorable for germination of *Sclerotinia cinerea* spores, and, consequently, about twice the usual amount (6) of pentathionic acid was required to prevent germination.

Plant Pathogenic Bacteria

Three species of bacteria—*Phytomonas pruni, Erwinia amylovora,* and *P. tumefaciens*—were used in the tests. Eighteen to 24-hour-old cultures were used because at this age the organisms were probably at their most active and vigorous stage of growth. The toxicity of pentathionic acid to these bacteria was tested by the Rideal-Walker method (9), modified in a few respects. Instead of only 0.1 cc. of culture 0.5 cc. was introduced into the solution the toxicity of which was to be determined.

The pentathionic acid for the tests was prepared from the barium salt by precipitating out the barium as a sulphate. The toxicity of phenol was determined by the same modified method. The following formula was used in calculating the phenol coefficient of pentathionic acid.

$$\frac{\frac{a_1}{b_1}+\frac{a_2}{b_2}}{2}=\text{Phenol coeff.}$$

In the formula, a_1 and a_2 represent the dilutions of pentathionic acid required to kill in $2\frac{1}{2}$ and 15 minutes, respectively, whereas b_1 and b_2 represent the dilution of phenol in similar tests.

TABLE 5.—*Toxicity of pentathionic acid to Phytomonas pruni, Erwinia amylovora, and P. tumefaciens; and the phenol coefficients*

Name of organism	Dilution of pentathionic acid necessary to kill		Phenol coeff.
	2½ minutes	15 minutes	
P. pruni	1–3,000	1–15,000	77.50
Er. amylovora	1– 500	1– 1,000	6.25
P. tumefaciens	1– 500	1– 2,000	11.46

The grams of the acid and the number of cubic centimeters of water necessary to make the dilutions that were required to kill the bacteria in 2½ and 15 minutes, together with the phenol coefficients, are given in table 5. The results of some preliminary tests indicate that sulphuric acid also is toxic to these bacteria. The toxicity of the sulphuric acid, however, is apparently due to the hydrogen ion, as, in tests in which buffered solutions were used, no toxicity was apparent. On the other hand, pentathionic acid exerted a toxic action 2 to 6 times greater than did sulphuric acid. It is concluded that the pentathionate radical of pentathionic acid is very toxic to these plant pathogens.[2]

Plant Lice

The experiments of O'Kane and Conklin (7) were called to the attention of the writer about the time similar work on fungi had been completed. These entomologists, in their studies on the toxicity of lime sulphur to San Jose and oyster-shell scales, found that there was neither enough hydrogen sulphide nor sulphur dioxide evolved from lime sulphur to kill the insects. The failure of these sulphur compounds suggested the testing of pentathionic acid.

Three common species[3] of aphids—the bean aphis (*Aphis rumicis* Linn.), the cucumber aphis (*A. gossypi* Glover), and the Spiraea aphis (*A. spiraecola* Patch)—were used in tests to indicate the value of pentathionic acid as an insecticide. Small plants or twigs infested with aphids were brought into the laboratory and set up in distilled water. In each test, the host and the aphids on it were thoroughly sprayed with either pentathionic acid, nicotine sulphate, or water. Potash fish-oil soap (0.5 per cent) was used as a spreader in all of the sprays.

About 24 hours after the application of the spray, counts were made of the aphids, those not responding to the touch of a needle point being considered dead. The numbers of insects, dead and alive, that dropped from the plants and of those that remained on the plants are given in table 6.

[2] The toxicity of the acid solutions, prepared by the writer, and of phenol was determined by R. C. Thomas, Department of Botany and Plant Pathology.

[3] Determined by C. R. Cutright, Department of Entomology, Ohio Agricultural Experiment Station, Wooster, Ohio.

TABLE 6.—*Toxicity of pentathionic acid and of nicotine sulphate to 3 species of plant lice*

Species of aphids and the materials tested	Dropped from plant		Remaining on plant		Dead
	Alive	Dead	Alive	Dead	
					per cent
Cucumber aphis on cucumber					
0.02 per cent nicotine sulphate	0	5	9	210	95.9
0.02 " " pentathionic acid	3	7	116	102	45.8
0.05 " " " "	3	12	17	197	91.3
Distilled water check	5	1	197	2	1.5
Bean aphis on Euonymus					
0.02 per cent nicotine sulphate	1	7	9	302	96.9
0.02 " " pentathionic acid	11	7	37	246	84.0
0.05 " " " "	2	14	7	302	97.2
Distilled water check	3	0	180	7	3.8
Spiraea aphis on Spiraea					
0.02 per cent nicotine sulphate	15	15	12	296	92.0
0.02 " " pentathionic acid	9	2	19	347	94.6
Distilled water check	13	10	217	12	8.7

Plants sprayed May 30, 1930, 10 a. m. Counted May 31, 11.30 a. m.

The results of these preliminary experiments indicate that pentathionic acid is very toxic to plant lice. It appears to be about $\frac{1}{3}$ as toxic as nicotine sulphate to the cucumber aphis and equal to the nicotine spray in the control of the Spiraea aphis.

The Effect of Pentathionic Acid on Earthworms

Some preliminary tests on the effect of pentathionic acid on earthworms were made by H. F. Dietz, Research Fellow, Department of Entomology. He found that a 0.5 per cent solution of the acid was capable of killing the worms in an hour. A reducing action of the pentathionic acid was indicated by the bleaching of the worms. He further observed that, from the standpoint of the time necessary to kill, the acid compares favorably with pyrethrum.

Colloidal Sulphur as a Fungicide

On the following pages are given the results of some preliminary tests on the effect of colloidal sulphur on the apple scab (*Venturia inaequalis*), the bitter rot of apple (*Glomerella cingulata*), and the damping-off of tomato (*Pythium debaryanum*), organisms. No conclusions are drawn from the meager results. They are given here merely to indicate some of the possibilities of hydrophilic colloidal sulphur.

Venturia inaequalis

Tests of the toxicity of pentathionic acid to the ascospores of *Venturia inaequalis* were the only ones in which the perfect stage of a fungus was used.

Small apple trees, growing in the greenhouse, were sprayed with colloidal sulphur varying in strength from ½ to 3 lb. to 50 gal. of water. On the following day leaves that had been thoroughly sprayed were brought into the laboratory, washed in a steady stream of water for 10 minutes, and then placed in the bottom of a Petri plate. An old apple leaf bearing mature ascospores was fastened by a wet filter paper in the top of the plate. The leaves were held in this position for 5 hours, during which time ascospores were discharged from the old leaf onto the sprayed leaf. The source of inoculum was then removed and the plate was set away at 23° C. for 24 hours. Apple leaves sprayed with dry lime-sulphur, 3 lb. to 50 gal. of water, and treated in the same way, were employed in control tests. At the end of the germination period the percentage of germination was determined, as described earlier.

TABLE 7.—*The toxicity of colloidal sulphur and lime-sulphur, sprayed on apple leaves, to Venturia inaequalis ascospores*

Tests	Types and concentrations of sprays and percentage germination					
	D.L–S. 3%	C.S. ½%	C.S. 1%	C.S. 1½%	C.S. 2%	C.S. 2½%
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	4.6	47.2	17.3	7.4	2.1	0.0
2	10.6	31.4	21.9	6.4	0.0	0.0
3	5.1	44.0	30.7	2.1	0.0	0.0

The results of 3 quadruple tests, given in table 7, indicate that colloidal sulphur, 1.5 lb. to 50 gal., is as toxic to ascospores of *Venturia inaequalis* as dry lime-sulphur, 3 lb. to 50 gal. It should be remembered that the sprayed leaves were subjected to washing for 10 minutes in a steady flow of water. In the tests neither of the 2 types of sprays caused noticeable injury to the apple leaves.

Glomerella cingulata

Leaves for this experiment were sprayed and treated in the same manner as in the preceding one, except that wettable sulphur instead of lime sulphur was used in the checks. After the leaves were washed, they were sprayed with a spore suspension of *Glomerella cingulata*. After 24 hours, no germination had occurred on the leaves sprayed with colloidal sulphur, 2 and 3 lb. to 50 gal., whereas on leaves sprayed with the same amounts of wettable sulphur over 50 per cent of the spores had germinated. Here, again, no injury to the apple leaves was apparent.

Pythium debaryanum

In an effort to control damping-off of tomato seedlings 3 tests were made. In one test the seeds were dipped in a heavy suspension of colloidal sulphur to which alcohol had been added. The seeds were then dried and planted in soil contaminated with *Pythium debaryanum*. In another test, the soil was thoroughly soaked with a colloidal sulphur suspension. After the soil was dry again, tomato seeds were planted in it.

Although the third test does not include the use of colloidal sulphur, it is included here because Babson (11) has shown that when sodium thiosulphate is applied to soil rich in organic matter about 80 per cent of it is transformed to pentathionic acid. On the basis of this, a 1 per cent mixture of sodium thiosulphate and soil infected with *Pythium debaryanum* was made up and planted with tomato seeds.

In all 3 of the tests the stand of tomato seedlings was improved by the respective treatments. There was some evidence of seed injury in the first and third tests, but it was not severe enough to be a limiting factor.

SUMMARY AND CONCLUSIONS

Sodium, barium, and potassium pentathionates were prepared from concentrated acid formed by the action of hydrochloric acid on sodium thiosulphate. These salts are stable when thoroughly dried and kept at room temperature. In the presence of moisture they break down, forming, among other products, sulphur and sulphur dioxide.

The optical properties of the barium and potassium salts agree with the descriptions and measurements of the pentathionate salts of the elements. The optical properties of the sodium pentathionate have been determined by V. H. Morris.

By gravimetric methods, the various salts were found to be from 95 to 98 per cent pure with reference to the amounts of the basic elements and the sulphur.

On standing, a concentrated solution of pentathionic acid slowly decomposed, forming free sulphur and sulphur dioxide. However, dilute solutions showed only slight decomposition after standing 6 months.

By volumetric titrations ,the acid prepared from barium pentathionate was found to be about 1 per cent trithionic, 3 per cent tetrathionic, and 96 per cent pentathionic.

A suspension of hydrophilic colloidal sulphur, prepared by the sulphur dioxide-hydrogen sulphide method, when reduced to a paste, was found to contain about 47 per cent free sulphur, 34 per cent pentathionic acid, and 19 per cent water.

Sulphur dioxide was formed when the paste was exposed to air. When sealed in an air-tight container, the product seemed quite stable. A spray suspension, 3 lb. of paste to 50 gal. of water, did not smell of sulphur

dioxide. In such a spray the sulphur remained in good suspension for 3 months.

Pentathionic acid was found to be toxic, to different degrees, to 9 pathogenic fungi, 3 bacterial pathogens, 3 species of plant lice, and to earthworms.

Hydrophilic colloidal sulphur was found to be toxic to 3 plant-pathogenic fungi. In consideration of the strength of pentathionic acid necessary to kill or inhibit growth of the organisms and the amount of pentathionic acid in the sulphur paste, the following deductions were made. A spray made up of 3 lb. of paste to 50 gal. of water contained 3 times as much pentathionic acid as was required to prevent germination of the spores of the bitter-rot fungus (*Glomerella cingulata*), 8 times the amount necessary to inhibit growth of the fire-blight organism (*Erwinia amylovora*), and 12.5 times the amount that killed 1 species of the apple aphis (*Aphis spiraea*).

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THE ANATOMY OF THE "KROEPOEK-DISEASED" LEAF OF NICOTIANA TABACUM AND OF ZINNIA ELEGANS

L. C. P. KERLING

(Accepted for publication May 4, 1932)

From Dr. T. H. Thung, phytopathologist at the Experimental Station "voor Vorstenlandsche Tabak" at Klaten, I received leaves of Kroepoek-diseased tobacco plants, used by him in his research on the symptoms and cause of this disease.

The diseased leaves have fantastically bent veins; Jensen (3, p. 51), who gives a short description of this disease, says that the leaf has the appearance "as of being kneaded and wrinkled in a hand, and again partly unfolded from itself." This is the reason that this disease was named "Kroepoek," after a well-known Indian dish. Many investigators have described it. For a complete list of literature I may refer to a paper of Dr. Thung, which will appear shortly.[1]

The cause of the Kroepoek of tobacco is a virus, which can be transferred from diseased to healthy plants by grafting or direct organic connection. The vector was discovered only recently almost simultaneously by Storey (8) at Amani in East Africa and by Thung at Klaten. It is a white fly of the family *Aleurodidae* and the genus Bemisia. The insects could induce the symptoms of Kroepoek on healthy plants in insect-proof houses.

Aleurodidae were also noticed on many weeds, but the identity of these with the ones on tobacco could not yet be settled. Many plants have disease symptoms coinciding with those of the Kroepoek: thus I found at Djokjakarta, in various gardens, zinnias with thickened and curled leaves.

Thung[1] transferred 2 white flies of a diseased zinnia to healthy tobacco plants in a greenhouse. After 14 days the tobacco had the Kroepoek disease. The same virus thus induces the symptoms of the disease on both of these plants.

The appearance of the Kroepoek-diseased leaves has been already repeatedly described by various investigators. A short repetition will here, however, not be superfluous, in order to connect definitely the macroscopic and microscopic symptoms.

MACROSCOPIC SYMPTOMS OF THE DISEASED TOBACCO LEAF

Thung[1] distinguishes 3 principal types, which produce constant symptoms by inoculation, independent of incidental circumstances. Also, in

[1] This paper will be published in *Mededeeling van het Proefstation voor Vorstenlandsche Tabak*. Dr. THUNG was so kind as to give me much information concerning the disease, for which I wish to express here many thanks to him.

transferring them by means of the white flies, each type retained its individuality.

Type a: the most common Kroepoek. The deviations are visible on both sides of the lamina. At the upperside the leaf sometimes exhibits over the whole length a downward-pointed nod due to the curving of the lateral veins of the first order. The midvein also may be curved. The ultimate branches of vascular network of the leaf are locally sunken, so that shallow furrows occur. On the underside of the leaf the curved veins present a fantastic course, their surface is irregular (Fig. 1, B). The more minute veins produce swellings to the end that even the most minute branches become thus involved. The swellings look like round or somewhat elongated, often irregular knobs and the original course of the vein remains visible in the thickening (Fig. 1, A). Sometimes the large and the small

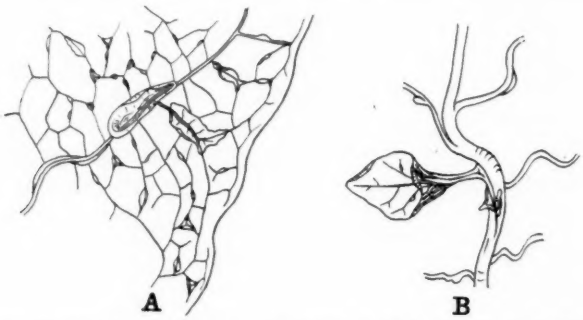

FIG. 1. Common Kroepoek of tobacco. A. Portion of leaf. B. Cup-like outgrowth of the main vein of a tobacco leaf.

veins form together a thickened network. The veins of the first order especially protrude like thick cords above the leaf. The swellings very often enlarge, mostly at both sides of a vein, so that they form a protruding margin, which is loose from the lamina. These margins stand oblique upwards, so that a second lamina, 2–14 mm. long, is formed, adjoining the original one and having with this a common vein (Fig. 1, A). The original lamina and the new one are in all cases situated with their undersides against each other as can be distinctly seen in the veins of the new, secondary lamina. The largest secondary leaflets are chiefly situated near the midrib and at the lateral veins of the first order. They are of very irregular form, sometimes somewhat oblong, mostly elongated along a vein until 18 mm. in length. The margins are irregularly lobed or crisped, often curled and sometimes locally grown together with the vein from which the leaflet issues. In a single case the leaflet transverses the vein. Often a second, smaller lamina develops under the new one, grown together at the

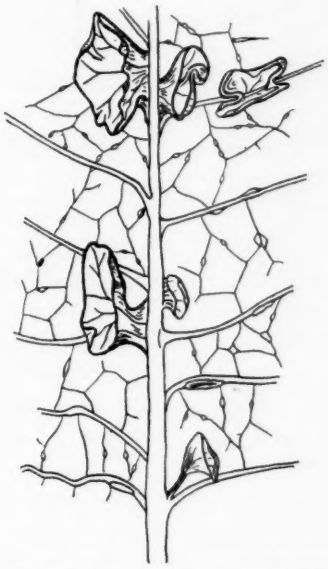

FIG. 2. Leaf- and cup-like outgrowths from the main vein of tobacco.

base and with the margins with the first one, so that a pocket-like appendix arises.

In the strongest deviated cases this distorted part elongates to a flat broad stalk on which a cup-like lamina is situated (Fig. 2). In the extreme cases the stalk is 1 cm. long, narrow, and supports a pocket-like lamina of a leaf form, like that of the original leaf (Fig. 1, B). The old and new laminae are connected only at the junction of the new "petiole." The new laminae have thickened veins similar to those of the diseased original leaf.

Type b: the curl disease. Especially the top leaves have regularly downward-curled margins, whereas the mesophyll between the veins protrudes convexly upwards. The main and lateral veins of the first order are not contorted or bent downward; the thickenings of the vein on the underside are more regularly spread over the whole lamina. This type is not further investigated here.

Type c: the clear Kroepoek. The margins of the leaf are turned upward. The main and lateral veins, which remain shorter than normal, have small thickenings "as if they are somewhat laced by a thread" (Thung). Some tertiary veins look somewhat transparent. Thickenings of the veins and secondary leaflets do not, however, occur here; this type differs from the preceding two.

TECHNIQUE

For the anatomical investigation fresh leaves were cut and also material fixed in alcohol was used. Diseased and healthy leaves of tobacco as well

as of zinnia were always treated in the same way; they were gathered at the same hour, the sections were treated alike. Some sections were treated with I. I. K., others with phloroglucin and HCl, with fuchsin or with gentian violet. Those treated with fuchsin were imbedded in Canada balsam, those with gentian violet in Hoyer's imbedding liquid.

THE "COMMON KROEPOEK," ITS MICROSCOPIC CHARACTERS

a. Portions of a diseased leaf, which show neither external nor anatomical changes. Contradictory to healthy leaves, the diseased ones gathered at 6 o'clock a. m. contain much starch in the spongy as well as in the palisade parenchyma. This phenomenon also occurs in leaves without distinct anatomical deviations.

b. Slight attack of the lamina. The sections were taken of portions attacked as shown in figure 1, A. Whereas the normal lamina has a uniform thickness of $\pm 150\,\mu$ throughout, the diseased one has at the underside irregular curvatures, and always where vessels are to be found. The thickenings may occur at the ends of those veins comprising only a few vessels and whose diameter is there $\pm 200\,\mu$. Under a small vein the normal leaf has 2 to 3 layers of spongy parenchyma cells of irregular form and with large intercellular spaces. The cells of the undermost row are somewhat more connected together and are elongated in the direction of the epidermis, $\pm 30\,\mu$ long and $\pm 15\,\mu$ high. The spongy parenchyma cells of the diseased leaf are more connected together, those of the undermost row are elongated at right angles to the epidermis, and are $\pm 30\,\mu$ high. Thus, a new palisade layer is formed, lower, however, than that of the upper side, which is $\pm 60\,\mu$ high. Through these changes the lamina curls upward, whereby the afore-mentioned sunken parts on the upper side are produced. The changes of the very small veins are in the main the same as those of the large ones.

c. Slight attack of the midrib and of the lateral veins of the first order. Leaves gathered at 6 a. m. contain a great quantity of starch in the medullary rays, the cambiform cells, in the cells of the pericycle and of the cortex parenchyma. Besides externally healthy looking portions of diseased leaves may already have anatomical deviations, which become more distinct in the neighborhood of the curled parts and of those bearing secondary leaflets.

The pericycle of normal leaves is, at the under side of the stele, not more than 2, at the utmost 3, cells wide. The primary phloem is situated in small groups at regular distances (Fig. 3, A). The diseased leaves have enlargements that arise through strong division of the cambium cells. While a cambium cell of the normal leaf does not form more than 1 to 3

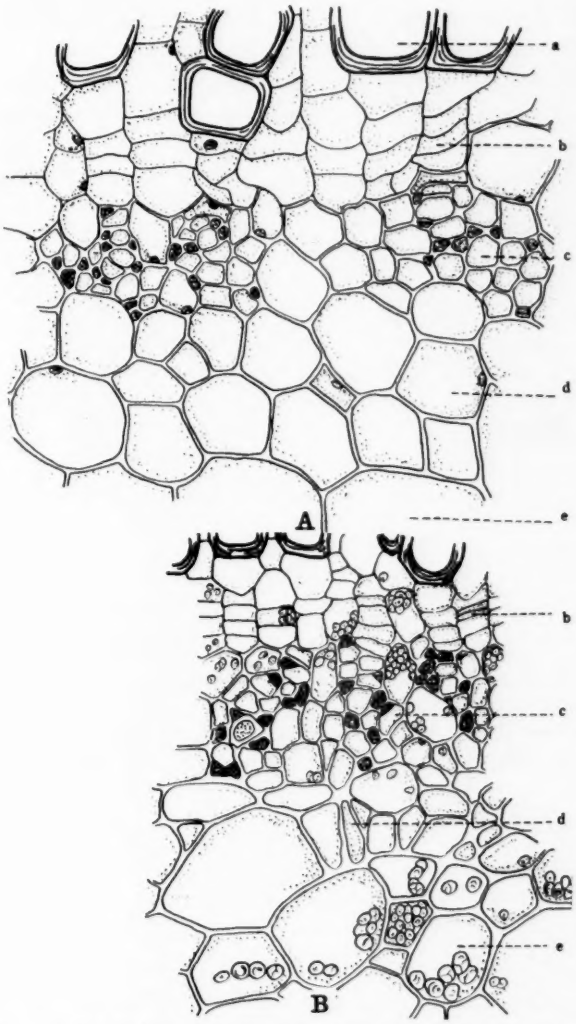

FIG. 3. Transverse sections of veins of tobacco leaf. A. From normal leaf: a, secondary xylem; b, cambium; c, primary phloem; d, pericycle; e, endodermis. ×295. B. From leaf showing beginning of common Kroepoek. ×210. Small letters have the same meaning as in A. However, the phloem was scattered, the pericycle was flattened, and the endodermis contained much starch.

rows of tissue at the outer side, the cambium of the diseased leaf forms radial rows of 5 to 7 cells. The small groups of primary phloem are no longer to be distinguished separately; they are united to complexes of irregularly placed sieve tubes and companion cells (Fig. 3, B). The sieve tubes are sometimes easily recognized by a sieve plate. The cells of the

pericycle also divide irregularly; small cells arise, some of which are strongly flattened. The endodermis of the diseased leaves remains easily distinguishable by the many large starch grains. Those of the healthy full-grown leaf are smaller; also, there are fewer cells with starch grains. At the upper side of the stele the intraxylary phloem behaves likewise; large irregular groups are formed. Because of the strong tension, schizogenous holes may arise in the pericycle (Fig. 4, A). There are here no other changes inside the endodermis.

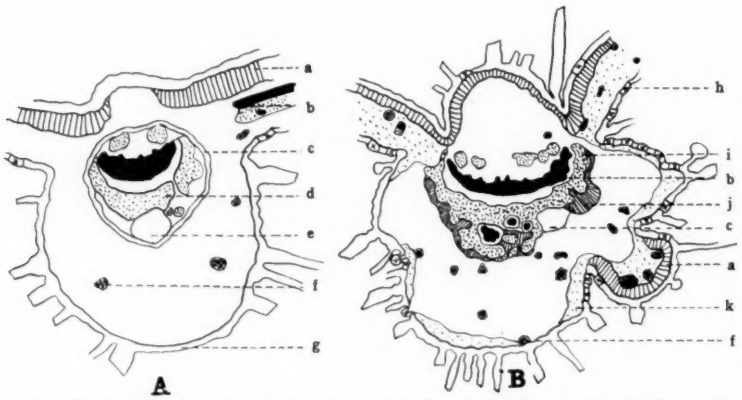

FIG. 4. Transverse sections of veins of tobacco leaf. A. Beginning of attack. ×295. B. Beginning of secondary leaflets. ×215. Small letters have same meaning in both parts of the figure. a, Palisade parenchyma; b, xylem; c, endodermis; d, primary phloem; e, schizogenous cavities; f, crystal cell; g, epidermis with hairs; h, stomata; i, secondary phloem; j, pericycle; k, spongy tissue.

d. Sections of a distinctly deviating part of the main vein. At the under side of the stele phloem proliferations are to be seen at one or more places, which may lead to irregular curving of the endodermis. Between and also within the proliferations, which consist chiefly of small cells with sieve tubes and companion cells, there remain strips of thin-wall tissue adjoining the medullary rays and the ends of the original cambium layer. The cells of these strips are irregularly situated; sometimes small rows of 2 to 3 cells occur.

In the midst of these proliferations small woody vessels arise without forming a distinct cambium. After some time circular cambia occur, producing new secondary xylem against the small woody vessels and outwardly forming secondary phloem. The primary phloem remains outside this. The cambia arise in the above-mentioned tissue strips (Fig. 4, B, and 5).

In a longitudinal section a distinct connection between the cambia of the old and of the new steles was sometimes observed, also the xylem parts may sometimes be connected by a series of small, narrow vessels. In a prolif-

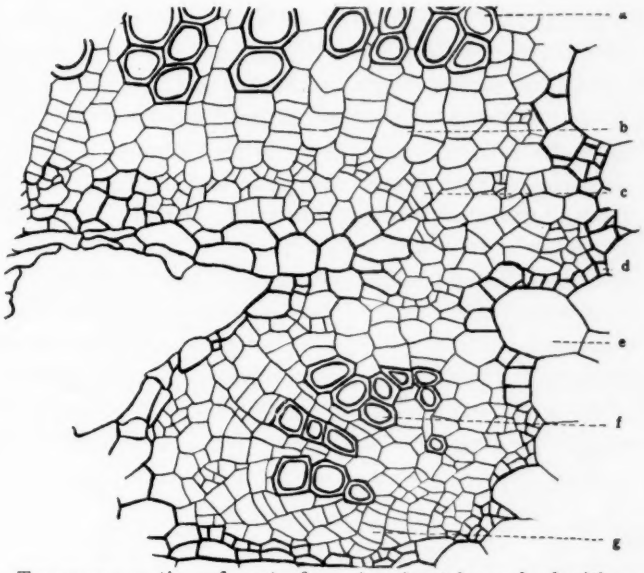

FIG. 5. Transverse section of part of a vein of a tobacco leaf with newly formed stele and schizogenous cavity. ×295. a, Secondary xylem; b, cambium; c, primary phloem; d, rest pericycle; e, endodermis; f, newly formed xylem vessels; g, newly formed cambium.

eration, however, the new strands have, for the greater part, a course independent of the old ones.

The new steles may becomes ± 160 μ in diameter. They remain entirely within the pericycle. As they increase they cause larger tensions in the surrounding tissue; nonproliferating cells of the pericycle are either flattened or torn (Fig. 5). In the latter case large schizogenous holes of

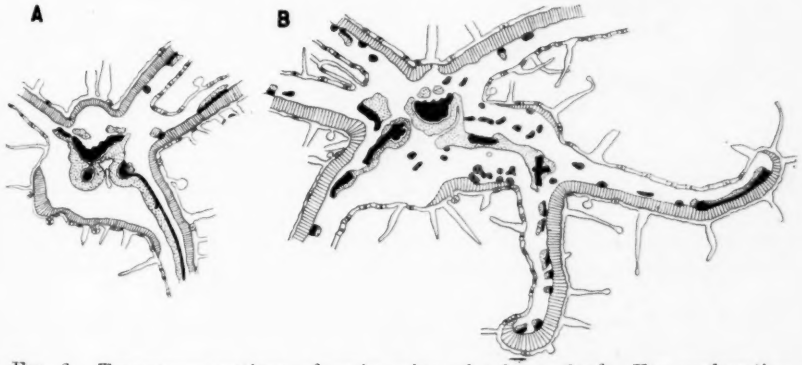

FIG. 6. Transverse sections of main veins of tobacco leaf. For explanation see figure 4. ×25. A. Slightly diseased. At the right there are 2 secondary leaflets. B. Severely diseased. At the left there are 2 loose and at the right 2 joint secondary leaflets.

irregular form arise. The endodermis is now very strongly transformed. If the new steles arise one-sided, then the midrib curves as described above.

The new steles are not always round; often the radial rows disperse flabellately from the point where the first and smallest vessels are found. The cambium of the steles situated at the sides of the vein opens and runs parallel to the original cambium of the main stele (Fig. 6, A). However, the new secondary xylem is formed here at the dorsal side of the leaf and the secondary phloem at the ventral side. In the latter case the secondary phloem adjoins the original primary phloem. In this reverse orientation the new stele proceeds towards the laterally rising secondary leaf (Fig. 6).

The first-formed primary phloem of the proliferations takes the place of the intraxylary phloem of the normal veins. In longitudinal sections the sieve tubes of the original primary phloem appear to have changed beyond recognition: division walls arise (Fig. 7, C). The sieve tubes

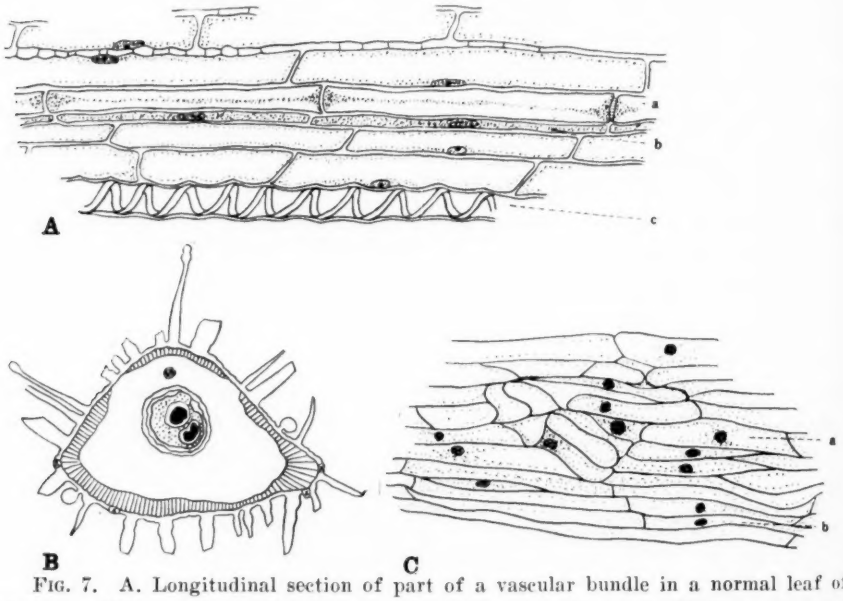

FIG. 7. A. Longitudinal section of part of a vascular bundle in a normal leaf of tobacco. ×295. a, Sieve tube; b, companion cell; c, spiral vessel. B. Transverse section of the stele of the cup-like outgrowth shown in figure 1, B. For an explanation see figure 4, B. ×115. C. Longitudinal section of part of a sieve tube in a new stele. ×450. a, Primary phloem; b, secondary phloem.

formed by the new cambium are elongated and narrow: 3 to 5 μ wide and ± 60 μ long. The sieve tubes of the normal leaf are ± 14 μ wide and ± 140 μ long (Fig. 7, A). The cortex parenchyma of a severely infested part has extended irregularly, especially at the under side where at both

sides sometimes two lobes have developed. They then form the macroscopically visible thickening of the veins between which the original course of the vein remains visible because of the unchanged median part. They are often more developed on one side than the other (Fig. 4, B). It also happens that only one lobe is situated in the median part or at the lateral side of the vein, which may be of very irregular form through the occurrence of folds. The lobes can develop into secondary leaflets that then obtain their above-described irregular arrangement. By the strong extension of the vein on the underside the parts of the original lamina form at both ends of the vein a more acute angle with each other than is the case in the normal leaf. Thus the above-described sunken parts arise. Furthermore, the cortex parenchyma of the upper side is often higher than normal, which probably causes the development of thickened veins on the upper side in the midst of a sunken part.

A normal vein has 5 to 6 rows of collenchyma cells adjoining the epidermis; the middle cells contain a few chloroplasts, those at the sides some more. The epidermal cells are here 30 to 40 μ wide; only a few stomata occur. They are situated in the same plane. The cortex parenchyma cells of the diseased leaf are unchanged, as is the number of crystal cells, but the layer of collenchyma is strongly reduced or has quite disappeared. Especially in the lobes, its place is taken by one or more rows of parenchyma cells containing many chloroplasts.

These cells are closely arranged; those of the outer row are elongated in a direction perpendicular to the epidermis. They form the transition to the palisade parenchyma that develops in the new lamina (Figs. 4, B and 6). Adjoining this tissue small vascular bundles are situated, sometimes at regular distances. They are in connection with the steles inside the pericycle and proceed later in the secondary leaflets. The epidermal cells have divided until they are only 8–12 μ wide. Especially at the lobes, many stomata occur. Sometimes the epidermis exists locally only of hair-like cells and stomata with their adjacent cells. The last ones are directed outwards, so that the stomata themselves protrude above the epidermis (Fig. 4, B). The substomatal cavities are larger than those of the normal leaf.

e. Development of the new lamina. If the two folds develop on both sides 4 new laminae arise. In most cases, however, only one fold on the left and one on the right side develop. Sometimes 2 laminae develop at one side and it may also occur that a lobe develops unequally so that it divides at some distance out on the midrib into 2 lobes. Thus the phenomenon of the stalked new lamina occurs (Fig. 1, B). If the splitting of the lobe takes

place only in the middle and the margins keep together, then the new lamina assumes the pocket or cup-like form, as described before.

The structure of the secondary leaflets is like that of the original lamina. They are, however, always situated with the similar tissues towards each other and towards the old lamina. The leaflets just under the original leaf turn the spongy parenchyma upwards, the palisade tissue downwards. The above described new steles, which are also situated upside-down, proceed in the leaflets and form the veins (Fig. 6, A). If a second leaflet lies under the first secondary leaflet, then it has again the position of the old leaf, and the veins are situated normally. In all cases the xylem part is turned towards the morphological upper side of the leaflet, the sieve part towards the under side.

MICROSCOPIC CHARACTER OF THE CLEAR KROEPOEK OF THE TOBACCO LEAF

Leaves gathered early in the morning are quite free from starch. In transverse sections the primary venation shows irregular swelling on the under as well as on the upper side. In these swellings the xylem is wider because of the marked division of the cambium that protrudes there. In longitudinal section this protrusion appears to be caused by the swellings of the ends of the cells of the xylem vessels (Fig. 8, A). The swollen ends of two cells are situated against each other. Also the secondary phloem is strongly developed. In contrast to the normal leaf (Fig. 3, A) a great number of sieve tubes and companion cells are here already formed (Fig. 8). The dimensions of these sieve tubes agree with those of normal ones. The primary phloem is flattened strongly, the sieve tubes are difficult to relocate in transverse sections. In longitudinal sections the cells are curved and often very narrow, except at the sieve plates. The walls are irregularly thickened (Fig. 9). The pericycle of the normal leaf is $\pm 60\,\mu$ wide; that of the diseased leaf $\pm 250\,\mu$, which is due to the enlargements of the cells up to $120\,\mu$ (Fig. 8, B). Between these strongly enlarged cells also flattened ones occur.

The gigantic cells possess thin walls; sometimes the wall is still thickened at one side. The smaller cells have thick walls; the intercellular spaces are filled with the same yellow substance as that of the cell walls. The endodermis is hard to find, while starch grains occur only sporadically.

The cortex parenchyma also shows local elongation of the cells in radial direction. Through this lengthening process other cells are sometimes flattened. In the enlarged cells division walls sometimes occur. The enlarged cells are often the most greatly swollen laterally. The cells turn these sides towards each other, so that local elevations are formed, causing the epidermis to protrude (Fig. 8, A). The result of this is the formation

of the irregular surface of the veins. The large cortical cells also are present in the veins of the second order and in smaller ones through which they obtain the "clear" appearance. The collenchyma in the midrib remains unchanged; it accompanies the epidermis, which covers the swellings. In the smaller veins the collenchyma has disappeared; also the epidermal cells may be somewhat enlarged.

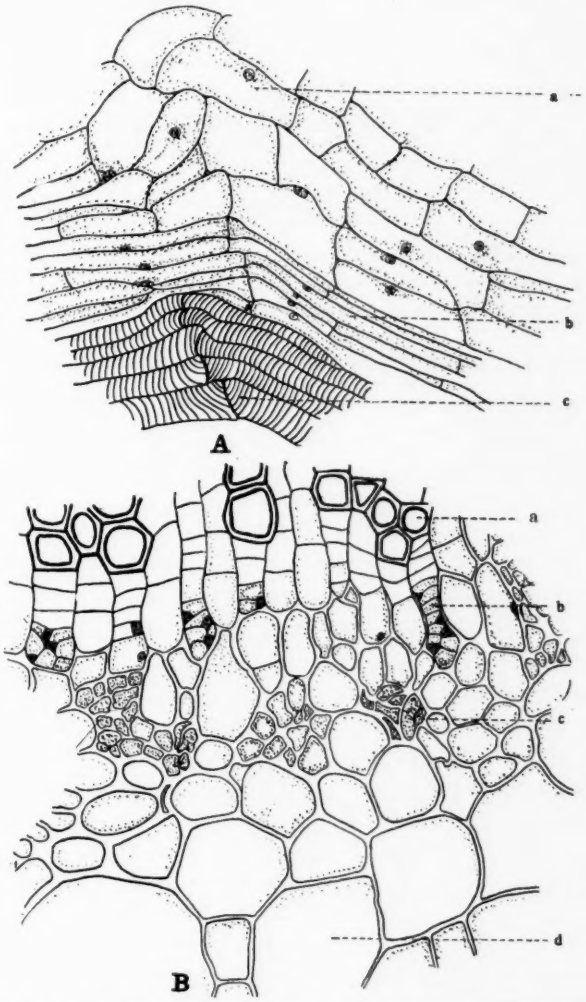

FIG. 8. Sections of veins of tobacco leaves with transparent Kroepoek. A. Longitudinal. ×115. a, Cell proliferation in the pericycle; b, secondary phloem; c, swollen cells of xylem vessels. B. Transverse. ×210. a, Secondary xylem; b, secondary phloem; c, flattened primary phloem; d, enlarged pericycle cells.

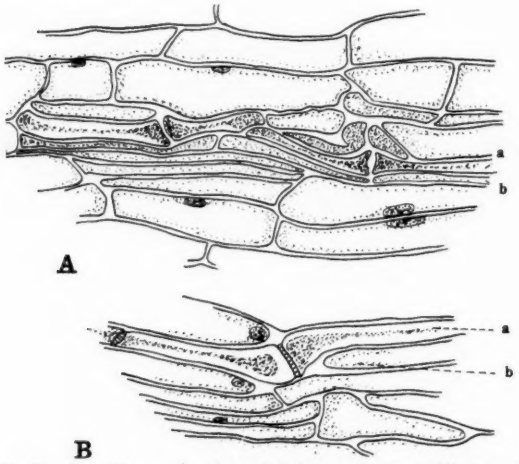

FIG. 9. Longitudinal sections of veins of tobacco leaves with transparent Kroepoek. ×295. A shows transformed sieve tubes of the primary phloem. B shows a swollen sieve tube. In both parts of figure: a, sieve tube; b, companion cell.

MACROSCOPIC CHARACTERS OF KROEPOEK-DISEASED LEAVES OF ZINNIA

The stems and leaves remain abnormally short; besides, the leaves are broader, thicker, and rougher. The cordate lobes are often so strongly developed that the lobes of a pair of opposite leaves are pressed against each other in folds and against the stem.

On the upper side the diseased leaves are lighter green than the healthy ones; sometimes only interveinal lighter spots occur. These parts protrude, then become convex upwards, while the dark green veins are sunken. This phenomenon is very striking, especially at the leaf base. The whole leaf becomes wrinkled.

On the underside the color of the lamina is a pale greyish green, sometimes silvery and shining. The finest veinlets and sometimes also some larger veins are less conspicuous than in the normal leaf, while they are sunken in the thickened mesophyll; they are situated in a furrow. In most cases, however, the larger veins are swollen, especially those of the first and second order. They lie then like thick cords on the lamina. Where the swellings develop locally or are laterally situated the veins take on a crooked course.

The disease symptoms can be found throughout the whole plant, sometimes also in the bracts of the involucre and even in the petals.

MICROSCOPIC CHARACTERS OF THE DISEASED ZINNIA LEAF

Whereas normal leaves (Fig. 10, A) are uniformly thick ($\pm 250\,\mu$), the diseased ones are irregularly swollen, especially in the veins, to a thick-

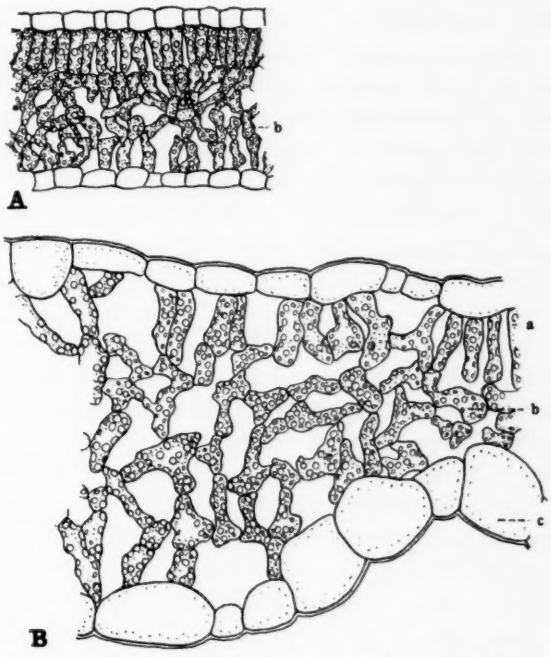

FIG. 10. Transverse sections of zinnia leaves. × 115. A. Healthy. B. Affected with Kroepoek. a, Palisade tissue; b, spongy tissue; c, epidermis. Note that in B the palisade tissue is spongy and epidermal cells are enlarged.

ness of ± 500 μ (Fig. 10, B). The epidermal cells of the normal leaf measure ± 30 μ, those of the diseased leaf are enlarged to ± 130 μ. The healthy leaves possess at the upper side 2 rows of palisade parenchyma cells with small slit-like intercellular spaces; under these occurs a loose spongy tissue of 3 to 4 rows of cells. The diseased leaf does not show much difference in structure between the under and the upper sides. The palisade parenchyma has more rounded cells than has the normal one; the cells are not perpendicular to the epidermis, but are situated obliquely and are separated one from another. Especially the cells of the second row have fantastic forms and are not to be distinguished from those of the underlying spongy parenchyma. The large intercellular spaces and epidermal cells give the leaf a light green color. Also, the spongy parenchyma cells are more dispersed and are larger than normal. Beneath a small vascular bundle the cells of the mesophyll are flabellately arranged; those of the diseased leaf separate and cause the semicircular swellings, as seen in section. The sections of the large veins are nearly square on the under side. The stele shows the same changes as those described for the common

Kroepoek of the tobacco plant; besides the original xylem and phloem numerous new steles, sometimes as many as 9, are to be found inside the pericycle. Also, here the primary phloem has increased strongly and the pericycle is enlarged through cell division. The cells are very small. Each stele has a ring-like cambium. In the vein, however, neither palisade parenchyma nor secondary leaflets develop.

In fresh sections sometimes inclusions of a brown granular substance occur. The particles are globular and are situated sometimes in, sometimes between the cells of the diseased vein.

SUMMARY

Both types of Kroepoek of the tobacco differ from each other not only externally but also anatomically, although they are transferred by the same Aleurodide. The permanence of two separate types in the infection experiments points to the fact that two different species of virus are concerned. The "transparent Kroepoek" produces in the veins typical swellings of the ends of the xylem vessel- and sieve-tube cells and of the cells of the pericycle and of the cortex parenchyma. The sieve tubes are curved: the cell walls of the primary phloem as well as those of the pericycle are irregularly swollen. Much secondary phloem is present, which still seems to function.

The "common Kroepoek" is so very different that it remains a question whether the type with the transparent veins may even be considered as Kroepoek. The common Kroepoek of the tobacco and the Kroepoek of the zinnia agree in many respects. That of the zinnia can therefore be transferred to tobacco by Aleurodidae. In the following points the anatomical deviations agree.

1. Disturbed transport of sugar in the whole leaf, even in the externally healthy looking parts.
2. Increase of the primary phloem in the veins.
3. Enlargement of the pericycle through cell division.
4. Formation of new woody vessels, surrounded by a cambium so that new steles arise inside the old pericycle.
5. Loss of the dorsoventrality of the leaf: In tobacco through formation of palisade parenchyma instead of spongy parenchyma; in zinnia through formation of a loose, irregular palisade parenchyma with large intercellular spaces.

Besides, palisade parenchyma and stomata are formed in the lobed veins of the tobacco; later, the lobes develop into secondary leaflets in which the new steles proceed in reverse orientation. The new leaflets lie with the morphological underside against the underside of the old leaf.

If more lobes are developed, then the similar sides of the leaflets are always directed towards each other.

Remarkable is this formation of a new palisade tissue and of new laminae. The increased assimilation can only augment the quantity of starch. The quantity of calcium oxalate is not changed; sections of diseased and of healthy veins contain an equal number of cells with crystals. This is in agreement with the experiments performed at the Experiment Station of Vorstenlandsche Tabak, which proved that the proportion of inorganic substance of the Kroepoek-diseased leaf remains unchanged (Thung).

The phenomenon of doubling of the lamina has long since been described. Morren (5) enumerates a number of cases in which secondary leaflets develop from the midrib, being directed with the corresponding sides towards the normal leaf. Schimper (7, p. 74) described a tobacco leaf with a stalked cup upon the midrib. Penzig (6) also mentions doubling of the lamina of tobacco with leaf reversion (Bd. III, p. 82); and he notes plants of other kinds with new formations on the midrib of the leaves both ventrally and dorsally, "welche entweder die Gestalt von flachen oder concaven, bandförmigen Blättchen, oder die von röhrigen, trichterförmigen, oben offenen, oft langgestielten Ascidiën haben." (Bd. II, p. 106). This description quite agrees with the common Kroepoek of the tobacco. The cause of these teratologies, named by Masters (4) under "Multiplication" and by Janse (2) under "splittingphenomena" is variously sought. Janse supposes a deviation of the growth enzymes. A too large quantity might cause local proliferation. However, he does not solve the problem. Also Penzig looks for the cause in growth substances, which might give rise to abnormal formations under influence of external stimuli such as parasites or feeding abnormalities. Also Jost (1), on account of experiments of Goebel supposes that in the first place feeding affects the symmetry of the leaf.

The question as to what is the cause of the teratologies is now in this case brought nearer to solution. The virus, transferred by the named Aleurodide causes the splitting of the tobacco leaf and also the loss of dorsoventrality of the zinnia leaf. However, this does not in the least explain the function of the virus in the plant. The first visible deviation is an accumulation of starch. Perhaps in connection with this is an abnormal behavior of the sieve tubes. Also it is possible that the virus influences the growth substances or the enzymes present in the tissues. Which part the accumulation of food plays on the formation of the new steles and on the laminae and thus on the local loss of dorsoventrality has still yet to be investigated.

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CYTOLOGICAL INVESTIGATIONS ON THE SPIKE DISEASE OF SANDAL, SANTALUM ALBUM

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INTRODUCTION

It is 35 years since the investigation of this disease, which has been estimated to cause a loss of nearly 6 to 7 lakhs of rupees in Southern India, engaged the serious attention of scientists and forest officers. Many theories had been put forward to explain the etiology of the spike disease of sandal, *Santalum album* L., by various workers, based on field observations, but investigations accurately carried out were first taken up by Butler (4) and Barber (2). A detailed description of the symptoms of the disease, both macroscopic and microscopic, was given by Coleman (5). Basing his conclusion on the evidence obtained from a large number of graft-infection experiments, he was the first to put forward the view that the spike disease of sandal may be of the nature of a virous disease. As opposed to this view, a few workers sought to elucidate the problem by the hypothetical consideration that the disease was the outcome of environmental factors. Hole (11) formulated an hypothesis that an "unbalanced circulation of sap," resulting from a variety of external factors, such as forest fire, unsuitable host plants, drought, etc., led to the outbreak of the disease. No doubt the importance of unfavorable external conditions as predisposing factors in relation to the appearance of the disease is universally recognized, but the significance of the term "unbalanced circulation of sap" is too vague for any plant physiologist to define. Howard and Howard (13), working with diseased peach trees, thought that improper grafting itself was the cause of the development of small leaves of spike and opposed the view held by Coleman (5) that spike disease could be transmitted by grafting. But this method of transmission has been tried by workers all over the world, as in the case of mosaic disease of hops, potato leaf roll, peach yellows, and other virous diseases, so much so that the objection cannot be taken as a serious one. In establishing the transmission of mosaic disease in hops, Thrupp (28) says "there is no evidence that the process of grafting can of itself in any way induce mosaic symptoms to appear in healthy susceptible hops."

[1] The writer wishes to acknowledge his indebtedness to Dr. Leslie C. Coleman, Director of Agriculture, in Mysore, for the encouragement and helpful suggestions given in the course of the investigation.

SPIKE DISEASE OF SANDAL, A VIROUS DISEASE

One of the recognized characteristics of virous diseases, in general, is that they are infectious and are transmissible by mechanical means. While some of the diseases, like the tobacco mosaic, possess a high degree of infectivity and are readily transmissible even at dilutions of 1–10,000, as shown by Allard (1), and are known to be filter passers, others among the group have not been found to be so highly infectious. In the case of sugar-cane mosaic, Brandes (3) found that the disease could be transmitted by rubbing the extract from diseased plants into punctures made in healthy plants, but the percentage of infection obtained was small. Sein (24), however, claims that 95 per cent of the plants were successfully infected when healthy sugar cane was punctured with fine entomological pins through a superposed layer of mosaic leaves. On the other hand, potato leaf roll, aster yellows, and infectious chlorosis of Abutilon, recognized as virous diseases, can be transmitted by grafting, but all attempts at transmission with the extract from diseased tissue have failed hitherto. Transmission of virous diseases by insects has been found in a large number of cases.

In the case of the spike disease of sandal successful transmission by means of grafting was observed by Coleman (5). It was noted that the disease was transmitted only in cases where the stock and the scion were organically united. Other methods, such as budding, leaf insertion (27), have also been reported to be successful in transmitting the disease. Inoculation of the disease extract into healthy plants has always failed to produce the disease symptoms. Punctures made with fine needles, the method adopted by Sein (24) for the transmission of sugar-cane mosaic, proved unsuccessful, but in one instance the plant began to develop mosaic symptoms. With our present knowledge, it may be assumed that the infective character of the spike disease of sandal is comparable to that of potato leaf roll and of peach yellows, which are transmitted only by grafting, warranting us in assuming that it is a virous disease. Cytological evidence to support this view, first put forward by Coleman (5), has been obtained by the writer's finding of intracellular inclusions in the tissues. Investigations on the nature of these intracellular inclusions are embodied in the present paper.[2]

HISTORICAL

Intracellular bodies have been known to occur in many of the virous diseases of animals such as vaccinia, rabies, lymphocystic disease of fish, contagious epithelioma, and sheep pox. Out of nearly 190 different plants, recognized by plant pathologists to be affected by virous disease, in only a

[2] A preliminary note on the subject by the writer was published in PHYTOPATHOLOGY (19).

few of them have the characteristic intracellular bodies been found. Simultaneously with the finding by animal pathologists of the Negri bodies in the hippocampus of dogs affected with rabies, Iwanowski (14) found in the tissues of tobacco mosaic amoebiform bodies closely attached to the nuclei, which he described as "parasitischen Amoeben erinnerende Plasmaanhäufungen." Goldstein (7) later made a careful cytological study of these bodies. Similar bodies, which the author described as X bodies, were found in the cells of Dahlia affected with mosaic and dwarf (8). Kunkel (15, 16) found intracellular bodies associated with the mosaic disease of corn, Fiji disease of sugar cane, Hippeastrum mosaic, and mosaic on Chinese cabbage and *Brassica pekinensis* (Hour). McKinney, Eckerson, and Webb (18) described inclusion bodies in the cells of rosette disease of wheat. Similar bodies were found by Smith (25) in the cells of Petunia, *Phytolacca decandra* L., *Euonymus japonica* L., and *Datura stramonium* L., affected by mosaic disease. Hoggan (10) found X bodies and striate material in a number of solanaceous plants affected with mosaic. In the pericyclic region of the young roots of strawberry dwarf plants, Plakidas (20) found that cells showing degeneration contained one to many amorphous intracellular bodies, staining black with iron alum haematoxylin. Similar structures were reported by Rawlins (21) in the pericycle of the root tips of sugar beets affected by curly top. Thus, it will be seen that inclusion bodies have been found in only 15 of the virus-affected plants.

MATERIALS AND METHODS

Material for the investigation was obtained from naturally infected areas in Uttarhalli, Bangalore, and Chamundi Hill, Mysore. The peculiar pendulous branches of spiked sandal, called the "willow type of spike," occurring at Hirehally, Tumkur, were also collected for examination. A comparative study was made of diseased material obtained by graft infection of healthy sandal plants. Leaves and petioles were fixed in various fixing solutions, such as weak Flemming's solution, Zenker's solution, Schaudinn's sublimate alcohol, and Carnoy's fluid. Sections, 5 to 7½ μ thick, were cut, and stained in Haidenhain's iron-alum haematoxylin and counterstained with anilin safranin or orange G in clove oil. Several other stains also were tried. Successful differentiation was obtained with Flemming's triple stain, picric methyl violet, and Goodpasture's carbol-anilin-fuchsin method. Likhité's (17) method of fixing in 2 per cent osmic acid and examining the sections without staining was not found suitable, as the precipitates formed interfered with the examination. Fixation with Flemming's weak solution was found to give the best results.

THE INTRACELLULAR BODIES IN SPIKED SANDAL

The cell contents were studied both in free-hand and microtome sections. Free-hand sections of fresh material were placed in picronigrosin for 24 hours and mounted in glycerine. The cell nucleus stained grey and the bodies yellow. Many vital stains were tried, but success was achieved only with a rapid staining in 0.5 per cent neutral red, the nucleus staining red and the body reddish yellow.

Microtome sections of spiked sandal leaves generally show the presence of intracellular bodies in almost every cell (Fig. 1, A and C). They are very prominent in sections of mature spiked leaves, in which much of the green chlorophyll has deteriorated. The bodies have not been found near the growing point and in the first pair of leaves next to it but can be distinctly made out in the 3rd pair of leaves. In an affected cell generally only a single body is present in close juxtaposition with the nucleus, but very often 2 bodies are seen adpressed to the nucleus 1 on either side. As many as 6 bodies have been observed surrounding the nucleus in some of the cells. Rarely the body is found free from the nucleus. In cases where more than 1 body is found in a cell, 1 to 2 of the large ones may be found adpressed to the nucleus, but the small bodies are to be made out scattered in the cell free from the nucleus.

In sections of spiked leaves, it is usual to observe in the palisade cells, that the nucleus and the body do not occupy a central place, but more often the nucleus is found wedged in between the body and the cell wall bounding the long axis of the cell (Fig. 1, A). Occasionally the body is found completely enveloping the nucleus.

As has been noted by various workers, in investigating other virous diseases of plants, the intracellular bodies in sandal spike are best made out when stained with Haidenhain's iron-alum haematoxylin. In leaf sections stained with this stain (longer method), the bodies stand the destaining with iron alum longer than the nucleus. Kunkel (15) in the case of Fiji disease of sugar cane, and McKinney *et al.* (18), in the case of rosette of wheat, found that the bodies were more easily destained with iron alum than were the nuclei, but Rawlins and Johnson (22) reported the reverse to be the case with the bodies found in tobacco mosaic. In sections of spiked leaf stained with Haidenhain's iron alum haematoxylin (shorter method), the destaining with iron-alum was more rapid in the bodies than in the nucleus. After 24 hours of staining the bodies were found to withstand the action of iron alum for nearly an hour. Counterstaining with orange G in clove oil brings out the structures in good relief. Counterstained with anilin safranin, the structural details in the bodies are better made out.

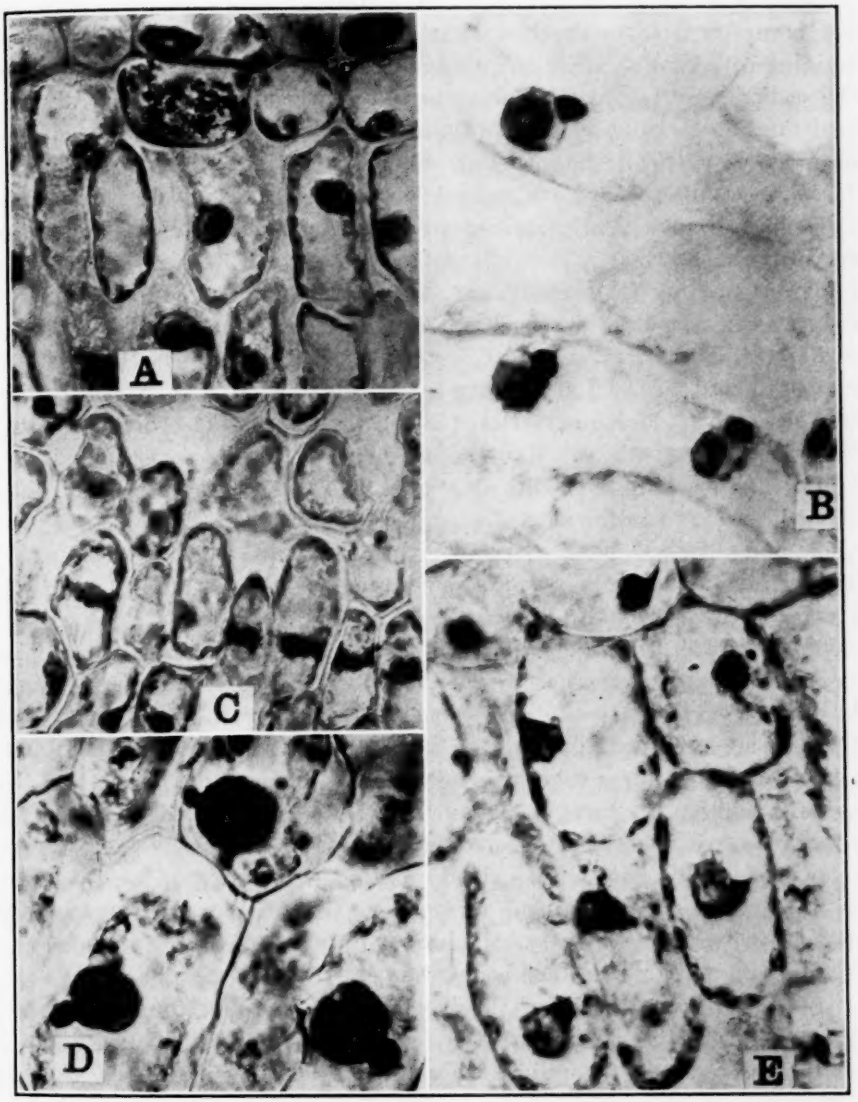

FIG. 1. Photomicrographs of sections of sandal-spike leaves. A. Deeply stained intracellular bodies attached to nuclei. ×820. B. Intracellular bodies with large central vacuoles and other small ones. The membranes around the bodies are evident. The black attached bodies are nuclei. ×980. C. Band-like intracellular bodies across the cells. ×740. D. Large-size intracellular bodies showing vacuolation ×980. E. Proliferation of bodies. ×820. A was stained by Goodpasture's method and B to E with iron-alum haematoxylin.

With Flemming's triple stain, the staining reaction of the bodies has not been found to be specific, as it seems to vary with the duration of staining with gentian violet and orange G. If, after staining with safranin, the sections are stained for a short period with gentian violet, rapidly carried through the higher grades of alcohol, and finally counterstained with orange G in clove oil, the nuclei are stained red; the bodies, orange yellow. If, however, the sections are stained longer in gentian violet, destained in alcohol, and subsequently stained with orange G in clove oil, the bodies retain the violet color. The different procedures adopted in the technique of triple staining by investigators, in general, may perhaps explain the difference reported by various workers in reactions of the intracellular bodies to this stain. Kunkel (16) reported that the bodies in Hippeastrum mosaic showed a special affinity for the orange G stain. The same reaction was observed by McKinney *et al.* (18) in the case of the bodies associated with the rosette of wheat. Rawlins and Johnson (22), however, found that, with the triple stain, the bodies associated with tobacco mosaic became pink. The bodies found in the case of sandal spike seem to react both to gentian violet and orange G, no special affinity being found for the latter stain.

The bodies are made out also by staining with dilute methyl violet followed by treatment with picric acid. This method was found to be successful by Schaffnit and Weber (23) in the case of the intracellular bodies associated with beet mosaic. Sections are transferred from distilled water to dilute methyl violet solution (59 parts of distilled water to 1 part of 1 per cent methyl violet), stained for 2 to 12 hours, rinsed in distilled water, allowed to stand for a minute in picric acid solution (80 parts saturated aqueous solution, 20 parts of 96 per cent alcohol), and dried between filter papers, cleared in xylol, and mounted in balsam. The body takes a grey color, while the nucleus takes a violet color. If the sections, after treatment with picric acid solution and removal of all excess water, are rapidly dehydrated with absolute alcohol, cleared in xylol, and mounted in balsam, the nucleus takes a deep blue color, the bodies a light violet one.

Sections of the spiked leaf also have been stained according to the technique followed by Goodpasture (9) for detecting the cell inclusions associated with rabies, and epithelioma contagiosum. They are stained, first, with carbolanilin fuchsin (0.5 gm. basic fuchsin; 100 cc. 20 per cent. alcohol, anilin oil 1 cc., phenol 1 cc.) for 10 minutes, decolorized with 95 per cent. alcohol, till the sections become pink, washed in water and counterstained with Löffler's methylene blue for 30 seconds, dehydrated in absolute alcohol, cleared in xylol, and mounted in balsam. Destained in 95 per cent. alcohol, the red color persists only in the bodies, even after all the other structures of the cell have lost color. The nuclei are stained blue, and the bodies, a cherry red to dull red. The bodies appear to have an affinity for fuchsin.

None of the previous workers on virous diseases of plants seems to have tried this staining method.

The bodies, associated with sandal spike, are generally round or oval. Often the outline of the body is wavy, with small pseudopod-like projections. In some cells the bodies are found extending from the nucleus, which is adpressed to the cell wall, to the opposite wall, thus appearing to form a band across the cell (Fig. 1, C). Cases have been observed in which the bodies have the appearance of a plasmodium, enclosing the nuclei in their folds. The presence of a membrane surrounding the body has definitely been noted. This is quite evident in cases where the contents of the body has shrunk (Fig. 2, B, body at extreme left). In some of the cells, owing to the disappearance of the contents of the body, the outer membrane persists as a ring attached to the nucleus. In certain instances, where the body presents the appearance of a mere mass of alveolar protoplasm enveloping the nucleus, the presence of a membrane is difficult to make out. Kunkel (16) found no evidence of a membrane surrounding the bodies associated with Hippeastrum mosaic, whereas the presence of a membrane has been reported in the case of the bodies of wheat rosette (McKinney, *et al.*, 18) and tobacco mosaic (Goldstein, 7). The bodies are generally composed of a recticulate matrix, containing one or many vacuoles of various sizes. There is either a large central vacuole surrounded by a thin layer of the body substance or, as is sometimes the case, there are one to two large vacuoles and numerous small ones (Fig. 1, B). As many as 11 vacuoles have been noted in some of the bodies (Fig. 1, D). Kunkel (15) observed in the bodies associated with the Fiji disease of sugar cane a process of division in a plane parallel to the short axis of the cell. Goldstein (7) found that the bodies in tobacco mosaic divide by constriction and the ones in Dahlia mosaic divided and are distributed by the divisions of the host cells. Likhité (17) also reported the occurrence of fission in the case of the bodies of tobacco mosaic. In sections of the petiole of the spiked leaf small bodies are occasionally seen that show a constriction in the middle, but it is impossible to say whether this can be considered a process of division. In addition, it is often to be observed in sections of some of the spiked leaves that the bodies show a large number of proliferations (Fig. 1, E). Figure 2, A, shows a body with 3 proliferations in focus. As many as 7 proliferations have been observed in some of the bodies. One is tempted to believe that the existence of tiny bodies distributed in some of the cells is due to this proliferation. These tiny bodies are stained black with iron-alum haematoxylin and bright red with carbol anilin fuchsin.

Measurements of the bodies along their greatest diameter show a variation ranging from 4.3 μ to 8.7 μ. The bodies were patiently watched under the microscope to see if there was any sort of movement as described by

other workers of mosaic diseases. Goldstein (7) found in the living cells of mosaic tobacco that the bodies changed their form and position frequently. In sections of spiked sandal, kept for a long time under observation, the orientations of the large vacuole or vacuoles in the bodies may be noted. Often the tiny vacuoles fuse together. Very rarely, in some instance a small projection is put forth slowly, which after a time is slowly withdrawn into the body, the entire process taking about 2 hours. It is, however, difficult to estimate correctly the significance of these movements. As Cowdry (6; p. 146) says, "the question always arises as to whether any movement seen, is their own, or that conferred upon them by mass movements of the cytoplasm." Mechanical strains, such as those induced by placing the cover slip over the section mounted in water, are likely to set up conditions other than those obtained in the living cell, which may perhaps result in evoking such phenomena.

THE DISEASED CELL

The effect of the disease on the contents of the leaf is very marked. Butler's (4) observation that the palisade cells elongate at right angles to the surface much more in the case of the spiked leaf than in that of the healthy one was not confirmed by Coleman (5). As the latter investigator pointed out, there seems to be no striking difference with regard to the size of the cells of the leaves of the same age, whether they be healthy or diseased. The writer's observations confirm this view, as will be made out from Figures 2, D and E. In young healthy leaves, the chloroplasts are found in abundance (Fig. 2, E). In old leaves also, these are to be made out lining the cell wall all around (Fig. 2, D). In spiked leaves, the chloroplasts seem to undergo a process of disintegration. During the early stages of the disease, the chloroplasts have a vacuolated appearance, showing clear spaces in their matrix. In very old spiked leaves, they appear as disorganized, shrivelled structures, adpressed to the cell wall. It is in such cells that the bodies stand out prominently. The presence of minute grains of starch in the leaves has been noted by Coleman (5). While, in the fibrovascular sheaths in the leaves, large starch grains are to be found, those found in the chloroplasts are minute. To make out the disposition of these grains within the chloroplasts, it is preferable to select the early stages of spike, as in the older leaves the chloroplasts have disintegrated. The starch grains are clearly brought out by staining with Němec's gentian violet method[3] and are stained bright blue, cytoplasm being grey. Eight to 11

[3] Sections of material fixed in Flemming's weak solution are placed from water in 2 per cent tannin for 30 minutes, washed for 1 minute in water, then transferred to 1½ per cent tartar emetic solution for 10 minutes, washed in water for 5 minutes, stained in 1 per cent gentian violet for ½ hour, rapidly dehydrated, cleared in xylol, and mounted in balsam (see Tunmann (29), 505–506. 29 pp.).

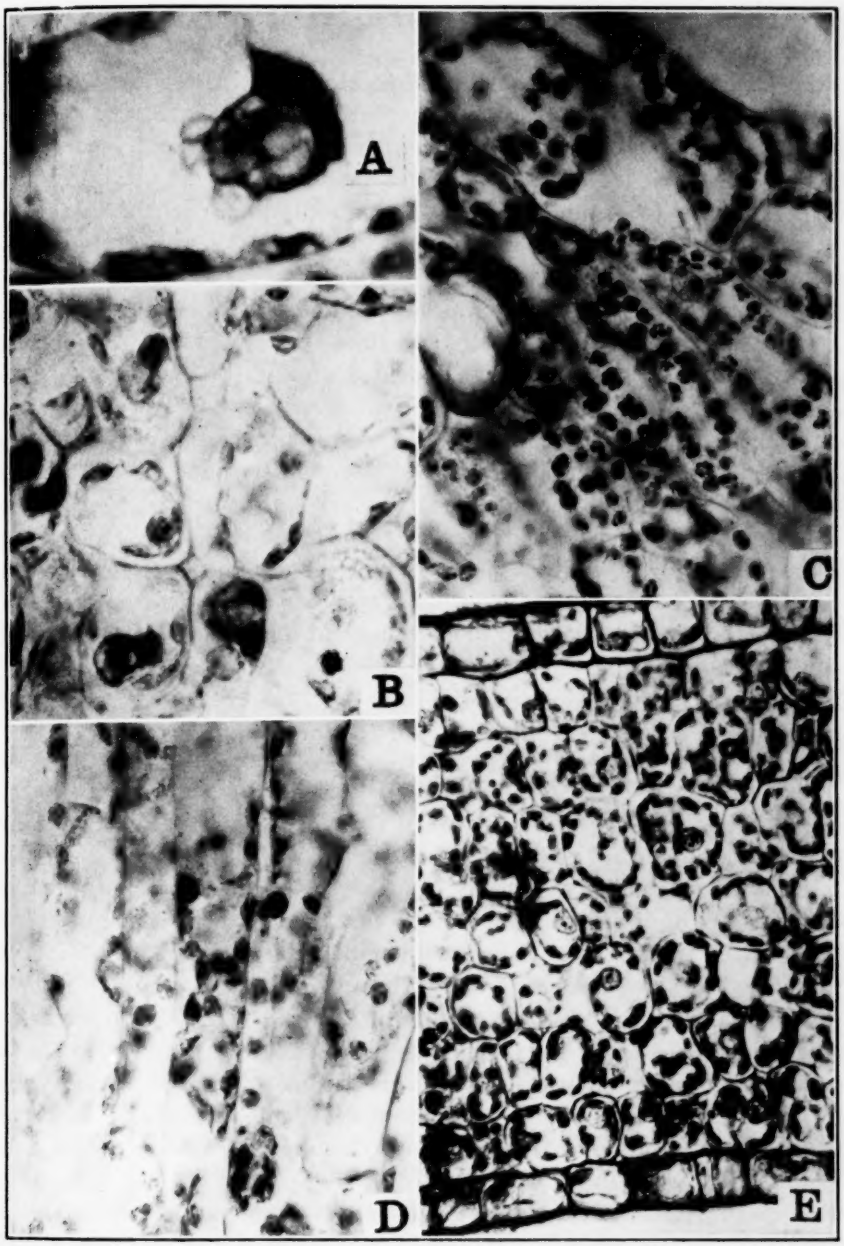

FIG. 2. A–C. Photomicrographs of sections of sandal-spike leaves. A. Highly magnified body with a reticulate structure from section shown in figure 1, E. There are three proliferations at the left. ×1,620. B. Intracellular bodies in the central region of the petiole. ×810. C. Minute starch grains in the chloroplasts. Stained with Némec's gentian violet. ×1,080. D and E. Photomicrographs of sections of healthy sandal leaves. D. Mature leaf, showing elongated palisade cells with nuclei and chloroplasts. ×1,080. E. Young leaf, showing nuclei and abundant chloroplasts stained with iron-alum haematoxylin. ×810.

grains have been found embedded in each chloroplast. Both round and rod shape grains have been noted in the same chloroplast (Fig. 2, C). In fact, they present the appearance of autochthonous starch formed generally in the chloroplasts of leaves; only, it is in a more intensified form. It is possible, as Butler (4) supposed, that the chloroplasts are stimulated by the virus to manufacture more starch than is necessary, the excess being stored in the parenchymatous tissues of the shoots.

The correctness of this view can be tested only after conducting experiments on the physiology of healthy and spiked sandal leaves. The nucleus in affected cells more often shows a dented appearance on the side where the body is attached. In a spiked leaf section some of the few cells in which bodies are not present have normal round nuclei, as in healthy leaf cells. On the other hand, some of the nuclei are unaffected, though bodies are found to be attached to them. In extreme cases, the nuclei present the appearance of crescent-shape, shrivelled up structures, recognized as such only by the presence of nucleoli. Nuclear degeneration in virus-affected cells has been observed in the case of vaccinia, where the bodies indent the nuclei. Kunkel (15, 16) also observed dented crescent-shape nuclei, in association with the bodies, found in the case of the Fiji disease of sugar cane and Hippeastrum mosaic. Goldstein (7), however, found that the nuclei were not affected by the bodies in the case of the tobacco mosaic.

THE NATURE OF THE BODIES

There has been much divergence of opinion in interpreting the nature of the intracellular bodies associated with plant and animal viruses. While some of the original investigators believed the bodies to be of the nature of organisms and classed them under the Chlamydozoa-Strongyloplasma group, later investigators have been inclined to the view that they are products of cell reaction produced by virous injury. Observations of movements in the bodies, and of the process of division, noted by Goldstein (7) and Likhité (17) in the cells of tobacco mosaic and by Kunkel (15) in the case of bodies associated with the Fiji disease of sugar cane, are evidences in favor of the view that they may be considered to be organisms. That the bodies consist of living cytoplasm, with chondriosomes in moderate numbers distributed in them, was established by Holmes (12) in the case of Hippeastrum mosaic. Henderson Smith (26), however, found that the inclusions, associated with *Solanum nodiflorum* infected with aucuba mosaic, were formed by the aggregation of small particles carried in the protoplasmic streaming and that they crystallize into forms that give a protein reaction. With our present knowledge, the intracellular inclusions associated with the spike disease of sandal, as in other viroses, can have only a pathognomic value.

SUMMARY

1. A cytological study of the spike disease of sandal was made with the result that intracellular bodies were found associated with this disease, similar to those described in other virous diseases.

2. Various staining reactions of the bodies have been tried. It was of interest to note that the bodies reacted in the same way as those found in animal virous diseases, such as rabies and epithelioma contagiosum, with Goodpasture's staining method, which, hitherto, has not been tried by workers on plant viruses.

3. Proliferation of the bodies was noted in some of the cells. The question whether the tiny bodies found in some of the cells are due to the proliferation of the large bodies is left open.

4. The effect of the disease on the cell contents was noted. The chloroplasts are stimulated to form an excess of starch. In the later stages of the disease, the disintegration of the chloroplasts sets in. When in association with the bodies, the flattened or dented appearance of the nuclei is of frequent occurrence.

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PHYTOPATHOLOGICAL NOTES

Dietrich Preferable Authority for Cronartium Ribicola.—Klebahn's[1] recent publication of Dietrich as the authority for *Cronartium ribicola* is of interest to Americans because the fungus is a permanent factor in North American forestry and because our extensive researches on the disease must be continued for an indefinite period. In 1909 the writer encountered the problem of the author who should be credited with the name *C. ribicola*. As it was impossible to find anywhere in North America one of the original specimens issued by Dietrich,[2] it was not known whether the label may have borne any sort of description that would entitle the name to a "legal" status. Arthur[3] stated that the name should be "*C. ribicola Fischer de Waldheim*," which was used for some time. On mentioning the use of Fischer de Waldheim to Farlow several years later he said that he was sure that this was incorrect, but he was unable to ascertain which Fischer was responsible for the name. After that time the name was given as *Cronartium ribicola* Fischer in all of our publications. Still later, in a personal search in European herbaria, the writer failed to find one of Dietrich's specimens. He, thereupon, concluded that settling the authority for *C. ribicola* must await action of some European investigator who might find a specimen. Some time ago Klebahn wrote suggesting that Dietrich ought to be the author of *C. ribicola* and urging the writer to try to clear up the matter. Again, however, it seemed impossible to do more than already had been done, and pressure of other more insistent matters led to this one being laid aside.

In the meantime much space had been paved with good intentions and Klebahn[4] had answered the problem himself. His method of attack was as follows: Dietrich's name technically was a *nomen nudum*, but he showed plainly that the fungus was a Cronartium and lived on Ribes. He then considered Fischer's[5] name and concluded (justly the writer believes) that the fungus was inadequately described to distinguish it from other species of Cronartium, *except for the fact that it lived on Ribes.* He then took up more recent and complete descriptions, like those of Ed. Fischer[6] and his

[1] Klebahn, H. Der Autor des *Cronartium ribicola*. Ztschr. Pflanzenkr. **41**: 209–213. 1931.

[2] Dietrich, H. A. Plantarum florae balticae cryptogamarum. Centuria 4, no. 21. 1855.

[3] Arthur, J. C. Uredinales. North American Flora **7**: 122–123. 1907.

[4] See footnote 1.

[5] Fischer. *Cronartium ribicola* Fischer nov. sp. Rabenhorst. Fungi europaei exsiccati. no. 1595. 1872; Hedwigia **11**: 182. 1872.

[6] Fischer, Ed. Die Uredineen der Schweiz. 590 pp. K. J. Wyss, Bern. 1904. pp. 433–435.

own.[7] He decided that all failed to distinguish *C. ribicola* from other species of Cronartium *unless the host were known.* Even then, on the same Ribes host, species like *C. ribicola* and *C. occidentale* can be distinguished only by biometric means or pedigreed cultures. He, therefore, concluded that, without knowing the pine hosts, Dietrich's simple statement that it was a Cronartium living on Ribes was as good as later descriptions and that he should be given credit for discovering, recognizing, and naming the fungus. The writer is heartily in sympathy with this decision, which he believes is a result of sound common sense and logic. Hereafter he intends to write the name *Cronartium ribicola* Dietrich.—PERLEY SPAULDING, Division of Forest Pathology, New Haven, Conn.

Comparison of Pycnial Stage of Cronartium Ribicola on Pinus Lambertiana and P. Monticola.—Studies were recently conducted at Daisy Lake, British Columbia,[1] to determine the relative susceptibility of western white pine, *Pinus monticola* D. Don, and sugar pine, *P. lambertiana* Doug., to white-pine blister rust (*Cronartium ribicola* Dietr.). One hundred and seventy-seven western white pines and 114 sugar pines were used for the test. These young trees ranged from about 6 in. to 2 ft. in height at the time they were planted, and some of them are now slightly over 5 ft. in height. Size of tree had no apparent influence on the production of the spore stages of the rust on either species.

During the course of the susceptibility studies a marked difference in pycnial production of the rust on the 2 hosts was noted. The following observations are based on 129 cankers on 42 infected western white pines and 179 cankers on 49 infected sugar pines.

On seedlings of western white pine the pycnia first began to appear about the latter part of May or the first part of June, reached their peak of production during July or August, and continued to develop generally until some time in October. They were produced in great abundance. The pycnial drops were very large and usually coalesced into continuous blotches or sheets. The pycnial scars were prominent rusty brown to bluish black spots.

On the sugar-pine seedlings pycnial production did not begin until some time in July or August and did not continue quite so late in the season as it did on western white pine. The pycnial drops were very small, usually widely separated and distinct from one another, and rarely coalesced. At

[7] Klebahn, H. Uredineae, in Kryptogamenflora der Mark Brandenburg. vol. 5a: 718–723. 1914.

[1] Lachmund, H. G., and J. R. Hansbrough. Preliminary report on the relative susceptibility of sugar pine and western white pine to blister rust. Jour. Forestry 30: 687–691. 1932.

no time during the summer were they observed on all cankers. The pycnial scars were generally indistinguishable and lacked color.

Generally, on western white pine the rust is prolific and abundant not only in pycnial production but also in aecial production. The aecial production on sugar pine was similar in this respect to that on western white pine, regardless of the meager amount of pycnial production.

The rust is not yet known to occur on sugar pine within its natural range. Consequently, the development and production of pycnia and aecia on this host as herein described cannot as yet be considered typical for the species. The production and development of pycnia and aecia on the western white-pine seedlings were typical of those observed on this species over a period of several years in numerous localities.—JAS. L. MIELKE, Division of Forest Pathology, Bureau of Plant Industry, Portland, Oregon.

A Correction.—In an article in PHYTOPATHOLOGY (A hitherto unreported disease of maize and beans. Phytopath. **22**: 637–644. 1932), a second article, by J. C. Haigh, (*Macrophomina phaseoli* (Maubl.) Ashby, and *Rhizoctonia bataticola* (Taub.) Butler. Ann. Roy. Bot. Gard., Peradeniya, **11**: 213–249. 1930), should have been included in the bibliography. The reference on page 641 to Haigh's inoculation experiments should relate to this article instead of the one cited. On page 642 reference to both articles should have been made in the following statement, "The pycnial stage, . . . found by Ashby and Haigh to occur in beans and sunflowers in Asia, . . ." Also, on page 642, the correct reference to Haigh's division of the sclerotial forms of *Rhizoctonia bataticola* into three classes is the 1930 article instead of the 1928 publication cited.—W. W. MACKIE, University of California, Berkeley, California.